THE EFFECT OF ALIEN GERMPLASM ON 2M UREA SOLUBLE PROTEIN ELECTROPHORESIS

by

ELYZABETH CHAVEZ

B.S. Universidad Central de Venezuela, 1973

A MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Food Science
Department of Grain Science

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1977

Approved by

Lynn S. Bates
Major Professor

Con mucho amor a mi madre.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Tables continued

INTRODUCTION

The world faces multiple and serious problems of food supply and distribution. This situation will get worse in the future, particularly with respect to protein resources. It has been observed that income levels influence the total protein consumption and the composition of the diet. When the income rises, a gradual change occurs from cheaper food (pulses, starchy roots, and grains) to more expensive ones (meat or animal products in general). In other words, a shift to sources of more and better proteins occurs. Under actual conditions, an improvement of the quality of life, socially and economically, does not keep pace with population growth and results in continual poor nutrition. It is our responsibility to solve this problem in some way. One solution is to expand the production of low cost protein crops, particularly cereal grains, which are the primary low cost protein producers. The proportion of cereals in various diets ranges from 70% in Pakistan to 17% in the U.S. (1). The value of cereal grains as food/feed and as commodities in international trade stimulates the continued investigation of the nutritional value of cereal proteins.

One can improve cereal protein quality to alleviate malnutrition by fortification of cereal-based foods with amino acids and/or protein concentrates. Nonconventional sources of protein for human consumption can also extend protein resources. However, the most permanent change in the supply of cheap protein is through genetic improvement.

We are concerned most with that third alternative: genetic improvement. It has been demonstrated to be an efficient method of overcoming protein deficiencies in cereal grains. At the same time it provides more nutrients with minimal possibilities of diet rejection in areas with strong traditional consumption of specific products.

Cereal genetic improvement is a time-consuming process and requires the collaboration of different agricultural sectors before widespread cultivation of "ideal cereals" becomes a reality. However, scientists all around the world are working toward improved cereal selection, and for the production of hybrids with better protein quality and quantity.

Hybrids, as man-made agricultural products, are some of the most far-reaching contributions of applied biology. Interspecific and intergeneric hybridization constitute possibly the most important factors in the history of cereals. They are accepted for their roles of providing genetic variability. Hexaploid wheat is one of the best natural examples; triticale the best man-made example. However, transferring genetic material across genera barriers is difficult and has limited the development of new man-made species. Recently developed hybridization techniques have been used to transfer genes between genera. Genes have been exchanged between species of wheat, rye, and barley (2,3). The concept is to selectively improve existing crops by using the best genes from other crop sources as well as increase the likelihood of producing totally new cereal species.

The objectives of this research were to investigate hybridization effects on prolamines, as environmentally independent markers, via starch gel electrophoresis and to demonstrate the transfer of genes between genera.

LITERATURE REVIEW

Wheat proteins

Wheat proteins have been studied for a long time: Every time a new technique is developed, it is applied invariably to the study of wheat proteins. Still, there are many unanswered questions in every study.

Osborne and Voorhees (4) divided the wheat kernel proteins into four classes according to their solubility as defined below

albumins: soluble in water

globulins: soluble in dilute salt solution, but insoluble in water

gliadins: soluble in 70% alcohol

glutenins: soluble only in dilute acid or alkali solutions

Fractionation on the basis of solubility has been the unifying key for most subsequent studies of cereal proteins.

Prolamines along with glutelins constitute storage proteins. Prolamines are located almost exclusively in the seeds of *Gramineae* as discrete protein bodies in endosperm cells. During fertilization two male nuclei from a single pollen grain enter the embryo sac. One of the sperm nuclei fuses with the two polar nuclei in the embryo to form the fusion nucleus, which develops into the triploid (3n) endosperm of the mature seed (5). Endosperm thus receives two sets of identical genes from the maternal parent and a single set from the male parent. The double maternal genome produces a quantitative effect on the protein components of the endosperm. In the immature endosperm tissue of wheat, gliadins are associated with the protein bodies which appear to be located in vacuolar structures (6).

Gliadins, classically defined as the protein fraction soluble in ethanol, are also defined as proteins ranging from about 50,000 to 80,000 molecular weight, sparingly soluble in water, but freely soluble in 2M urea solution or diluted acid (7). With respect to composition, they are characterized by their high content of glutamine (38- to 48% of all amino acids residues) and proline (15- to 30% of all amino acids residues).

Mosse (8) gave the prolamine percentage for some cereals (Table 1).

	Prolamine	
Species of Graminae	Name	% of seed protein
Wheat	Gliadin	45-60
Rye	Secalin	40
Barley	Hordein	40
Oat	Avenin	12
Maize	Zein	50
Sorghum	Kafirin	60

Table 1. Cereal prolamine content [Mosse (7)].

Almost half of the protein of those seeds are prolamines. The high content of poor nutritional quality prolamines in cereal grains, as evidenced by their amino acid composition, justifies the study of prolamines and the efforts to improve their nutritional quality and/or to minimize their synthesis with respect to other storage and metabolic proteins. Gliadins, have presented problems for the separation of individual components due to several factors: great similarity in physical and chemistry properties; small number of charged groups, high level of glutamine residues (9,10), and strong tendencies to aggregate through intermolecular bonds (11).

Physicochemical studies of gliadin have been carried out in: alcoholic solution (12,13), aqueous sodium salicylate (14), dimethyl formamide (15),

neutral salt solution (16), aqueous urea (17) and acid buffers (8,18). In general, fractionation methods lack sufficient resolving power, or nonideal conditions made the results difficult to interpret.

The first significant advance in the separation of gluten protein components was reported by Jones, et al. (18) who used the Tiselius free boundary electrophoresis and showed that symmetrical patterns could be obtained by the use of low protein concentration in conjunction with an aluminum lactate-lactic acid buffer system of low pH and low ionic strength.

Elton & Ewart (19) first reported the application of this buffer system to the electrophoretic separation of cereal proteins in starch gel; the only acceptable method of recognizing and separating material which was not soluble in aqueous solutions (20,21).

Woychick, et al. (17) using aluminum lactate buffer containing 3M urea revealed that gliadin was heterogenous and composed of 8 components which were classified according to their mobility in α, β, γ and ω regions. Today it is known that each fraction observed by Woychick is composed of a great number of proteins differing in composition. Combinations of sophisticated methods of extraction, isolation and purification have shown that gliadin consists of over 40 components (7).

The preparation of gliadin has often involved also the following treatments: heating to inactivate enzymes (17), application of high shearing forces to disperse mixtures (18) and extremes pH (22).

Because gliadin exhibits a strong tendency to aggregate through intermolecular bonds, the imposition of small charges on the protein or the addition of urea in an amount to yield a one to two molar solution will carry the gliadin into molecular solution. Increases in ionic strength

lowers the efficaciousness of the imposed charge and reverses the dispersion tendency in favor of the intermolecular attraction existing between the protein molecules (11). Under ionic strength of 0.001 and a concentration of 1%, gliadin has shown a large solubility (23).

The types of interacting forces operating between the protein molecules in solution includes hydrogen bonding, electrostatic bonding and dispersion or Van der Waals association forces. Urea presumably brings about physical rather than chemical changes in protein molecules. Molecular kinetic properties of gliadin can be studied in urea solution with minimal interferences from intermolecular aggregation (11).

The possible denaturating action of urea on gluten protein components is negligible since the intrinsic viscosities (24) and helix content (25) in urea free solution are not much different from those in 3M urea solution at the same ionic strengths. Also, there is no influence of urea (3M) on the ionic strength, osmotic pressure, optical rotatory dispersion, intrinsic viscosity, and sedimentation velocity measurements (26).

Urea is added to the gels to dissociate protein, to increase the rigidity of the gel and to sharpen band separation without obvious alteration of the protein (17,27). It also lets one work with higher concentrations of protein (18).

In starch-urea-gel electrophoresis, ammonium cyanate may be present in such gels. Cyanate in urea solution presumably carbamylates amino groups and thus alters the mobility of some proteins either in starch gel and/or polyacrylamide gels (28). The limits of this type of reaction depends on the accessibilities or reactivities of the amino groups involved. Cole, et al. (28) showed that protein patterns were only affected slightly due to urea addition and only then above pH 7 was the formation of cyanate troublesome. In acid medium the cyanate is decomposed and hence

not a important factor.

Urea is considered a hydrogen bond breaking reagent; it competes for hydrogen bonding sites that stabilize the gliadin structure as well as hydrogen bonding sites responsible for intermolecular interactions (9,11,29).

Lee, in 1968, (30) reported a method for gliadin preparation from flour by urea extraction. He confirmed, by gel filtration, that the protein present in urea solution was gliadin of low molecular weight and free from the high molecular weight glutelin.

Genetics of gliadin

Homoeology between the genomes and chromosomes of wheat and their relatives involves similar DNA sequences between the individual genes. Of course, the homology between several genes must be deduced from their amino acid sequences. Unfortunately, amino acid sequences have not been determined for many plant polypeptides, and we must depend on less direct measures such as the net electric charge of a molecule, which can be measured by its migration velocity in an electric field--for instance via starch gel electrophoresis (31).

There is evidence that proteins differing by a single amino acid residue, as a result of a single gene mutation, may exhibit different electrophoretic mobilities (32). Therefore, electrophoresis of a crude extract having a mixture of proteins will produce a pattern of bands characteristic of the species and the homology between bands will produce a theoretical estimate of their genetic similarity (31).

Using this criteria, Barber, et al. (31) by means of electrophoretic zymogram established a comparative genetic relationship of Triticinae chromosomes. Evidence of the transfer of alien genetic material to wheat (33), identification of genomes of allopolyploid species and their

putative genome donors (34); the confirmation of the current concepts of evolution in the wheat group (34,35,36) and the recognition of extraspecific or extrageneric characters (33), and various species relationship (31) have been achieved by use of starch gel electrophoresis, mainly on seed storage proteins.

The additive nature of proteins has been demonstrated in different Triticale strains (34,37,38), in a spontaneous amphiploid of Stipa and Orizopsis (39) and in a reconstituted hexaploid derived from 'Tetracanthatch' x Aegilops squarrosa hybrids (40). On the other hand, Barber et al. (41) detected a hybrid esterase band in Triticale not present in either of the parents. Yong & Unrau (42) found that the pattern of Triticale is a rough addition of the patterns of Triticum durum and Secale cereale, but reported also four hybrid bands, which led them to conclude that some interaction between genes caused the protein synthesis in the hybrid to differ from that of the parents or genome donors.

It has been shown that hybrid molecules from dimeric esterase enzymes of maize produce hybrid bands in the electrophoretic pattern (43). Kruse (44) reported that a summation of the parental protein complexes is observed in the hybrid protein of a cross between wheat x oat, even when true fertilization was not achieved.

Gliadins appear more suited for comparing genotypes within species. In fact gliadin composition, as determined by starch gel electrophoresis and column chromatography, is independent of environmental conditions and of grain protein content, and is controlled only by genetic constitution (45,46). However different varieties exhibited appreciable different gliadin patterns (47). This conclusion was confirmed also by

protein mapping, a combination of gel electrofocusing and gel electrophoresis (48). Hordein, barley prolamine, is also independent of growth conditions (49,50).

Shepherd (51) was able to correlate the changes in protein phenotype, counted as the absence of particular electrophoretic bands, with either increased or decreased dosage of known chromosomes. He showed that 10 of the 18 major bands present in Chinese Spring are controlled by individual chromosomes of homoeologous groups 1 and 6. Four of the remaining bands presented variation in staining intensity with certain nulli-tetra stocks. It seemed that two chromosomes pairs were involved in the control of each of these bands. Boyd, et al. (40) gave evidence that at least four seed proteins in Triticum aestivum are controlled by the short arm of chromosome 1D.

Few other cereal grain prolamines, such as hordein from barley and secalin from rye, have been studied due possibly to their lesser economic importance. Thus, there has not been any attempt to establish varietal differences according to electrophoretic patterns.

Barley prolamines have been characterized by using Tiselius electrophoretic technique (50), starch gel electrophoresis (53,54,55,56), continuous carrier free electrophoresis (57) and disc electrophoresis in polyacrylamide gel (54,59,60,61). Rye prolamines have been studied often in conjunction with gliadin, due to their relationship with Triticale proteins (37,38,41,47,51).

Dispersability in urea for barley protein (46.7%) has been reported to be lower than the dispersability for wheat and rye proteins (67.9 and 66.2%, respectively) (62). Hordein has been separated into six components. There is one fraction in hordein which aggregates reversibly in urea solutions (57).

Comparative studies of total wheat, barley and rye proteins are numerous. From the amino acid analysis of flour, rye and barley, one can show similar overall distribution of ionic polar hydrogen and hydrogen bonds (55). Wheat and rye present similar fingerprints which led some workers to conclude that their aminoacid sequences for some portions of their polypeptide chains are similar (62).

Recent research efforts have given a good example of the potential of genetic improvement in both protein quantity and quality. Genes with significant effect on lysine and tryptophan containing proteins have been identified. High lysine corn (opaque-2 modified, opaque-2, floury-2), high protein oats (Avena sterilis), high lysine barley (Hiproly and Riso 1508), high lysine sorghum and triticale, with higher protein and higher lysine than wheat, have stimulated interest in genetic improvement of crops specially when the results have been of immediate utility (64,65,66,67,68,69,70,71,72,73,74) and are integral to traditional food plants.

It is often difficult to achieve a combination of high yield, high protein and a good balance of amino acids in a particular genetic background (75). In fact there is generally a negative correlation between total protein and lysine content except for some varieties of oats and triticale.

A great deal of research has been focused on the improvement of cereal protein primarily toward increasing the level of lysine (76,77). The successful development of opaque-2 and floury-2 maize is a consequence of the identification of single recesive genes. It is possible to predict that genetic manipulation may help to develop a variety with a high content of protein and lysine. To improve the protein quality of any plant requires an understanding of the genetic control of protein synthesis,

the nature and the mechanisms by which genetic information can be translated into a particular sequence of amino acids. The conservation of a given set of amino acids sequences is favored by the specificity in its genetic control; addition or deletion of a nucleotide will produce changes in the reading frame (78). Thus, from the point of addition or deletion, a new set of codons is generated resulting in a completely different amino acid sequence for the protein. That could make the protein non-functional. For example, albumins with important metabolic function have indispensable sequences and structural conformations which must fulfill precise conditions. In the endosperm or storage proteins with no specific function, the overall amino acid sequences can be changed without affecting the plant (55,76).

There is also evidence of very complex excision-repair mechanisms in eukaryotes which involve a great number of physical and enzymatic reactions. DNA polymerasas, ligases, endonucleasas, transferases are involved in this type of mechanism (79). On the other hand; even when several genes are known to be involved in determining protein quality, we still do not know (as in Triticum) the exact number or the nature of these genes in terms of their recessivity, dominance or interactions (80).

Some understanding of genetic control of the synthesis of gliadin has been accomplished (81,82). Control is directed by the group 1 and group 6 of chromosomes of each of the 3 genomes on bread wheat (51).

Wide hybridization

The present success of Triticale, a wheat x rye cross, is stimulating the interest of plant breeders and geneticists to attempt other interspecific and intergeneric crosses between man's most important agricultural crops to improve our present food reserves.

The relative relationship among some members of the grass family has been elucidated through cytogenetic studies (Figure I) (83). Bendich and

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.

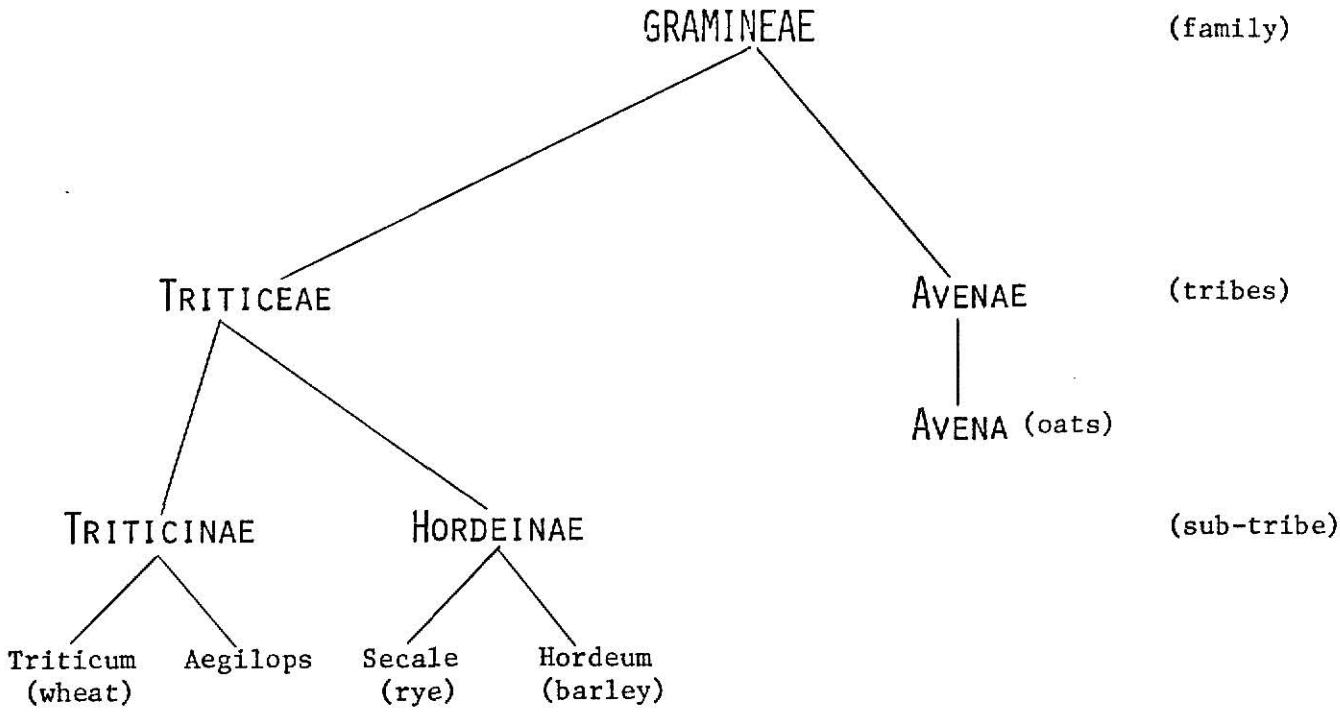

Figure I Taxonomic relationship among Gramineae

[Bell (83)]

McCarthy (84) compared DNA between some Gramineae establishing that rye is much closer to wheat than barley. Similarities between wheat and rye have also been shown by immunoelectrophoresis (37). Many attempts to cross wheat, barley and rye have been made since 1900 based on their economic importance, their believed common ancestry, as defined above, and the possibility of genetic recombination. Those have been the main factors taken in consideration to cross those genera (2,85-96). Many of the trials failed; few have been successful (2,96-99). In general, fertilization occurs but embryos abort due to abnormal endosperm development. In other cases progeny were produced but were sterile.

Bates, et al. (2,100) were able to obtain hybrids from durum wheat x barley; bread wheat x barley and barley x rye crosses, by using the stereospecific inhibition reaction hypothesis (101) to explain the factors of incompatibility existing between different species. This type of mechanism was suggested to be similar to immunochemical mechanism in animals. Control of such crossability barriers via animal effective immunosupressants was suggested and described (2). Chloroamphenicol, E-aminocaproic acid, gentisic acid, salicylic acid, and acriflavin are examples of the immunosuppresants used which were able to disrupt maternal wheat crossability barriers and to produce apparent paternal gene transfer from barley and rye without visible chromosome alternations. The phenyotypic characteristics of the progeny are constant and nearly identical to the maternal parent. Cytological abnormalities were not observed in later generation which suggested some kind of gene interaction. Segregation for plant height was observed commonly (98,99).

An investigation of this material for clues to the type of hybridization that occurred began with a multidisciplinary approach through proteins, morphological characteristics, secondary plant markers, disease

reactions, and other observations. Starch gel electrophoresis of prolamines, the research described herein, was used to follow protein segregation as one facet of the total investigation.

MATERIALS AND METHODS

The plant material has been already described by Bates et al. (97,98), as progeny with nearly identical morphological and cytological characteristics to the maternal parent but segregating for several specific characters.

The plant material used was the following:

Parental varieties

Triticum durum var. Cocorit 71

Triticum aestivum var. Tobari 66

Hordeum vulgare var. Apizaco

Hordeum vulgare var. Promesa

Hordeum vulgare var. Porvenir

Secale cereale var. Snoopy

Hybrid Progeny

F_5 materials from:

Triticum durum x *Hordeum vulgare* (vars. Promesa and Apizaco)

Triticum aestivum x *Hordeum vulgare* (vars. Porvenir and Promesa)

Hordeum vulgare (var. CM67) x *Secale cereale*

Analytical Procedure

The electrophoretic procedure is similar to the one described by Wrigley and McCausland for testing cereal grain prolamines (102).

Preparation of sample:

Grains were ground in a Wig-L-Bug dental amalgamator (Crescent Dental Mfg. Co., Chicago, Ill.)

Extraction of grain proteins:

Different solvents were used to probe their suitability to the scope of the research. Among the solvents used were:

Aluminum lactate buffer with and without urea

Urea (2 M)

Urea (8 M)

70% Ethanol

Water

Acetic acid

All the solutions were made up immediately before use. After extraction for different periods of time (from one minute to overnight) the mixtures were centrifuged at 7700 r.c.f. for 20 minutes to give clear extracts.

Separation of the proteins:

Electrophoresis was carried out on starch gel buffered at pH 3.1. Buffer used was prepared from aluminum lactate adjusted to pH 3.1 with lactic acid to a final concentration of 0.1122M and 0.5 mmho of conductivity (Conductivity bridge Model RC 16B2 k=1.0 3401 cell Yellow Springs Int.).

Buffer (450 ml) was mixed with urea (60 g) and starch (60 g) (Connaught Hydrolyzed starch, Connaught Laboratories, Ontario, Can.) in the same order as mentioned before. The mixture was heated with vigorous shaking until it became thick and viscous. After thickening the mixture was heated over a bunsen flame. Each 2 minutes the flask was left unagitated to remove air bubbles. This process was repeated several times until the temperature reached 185°F and the starch became nearly transparent. Foam was removed with a spatula.

The hot mixture was poured into the tray of the gel electrophoretic apparatus. The bottom piece of the tray and the surface of the cover

which are in contact with the gel were coated with mineral oil in order to avoid adherence of the gel to the tray. The cover with the slot former, was inserted carefully into the gel preventing formation of air pockets. The gel was allowed to cool overnight at room temperature. After the starch set, the cover was removed and the slots cleaned from any remanent oil. Samples (50-75 µl) were placed in each slot with a 1 ml syringe to avoid overfilling which could cause mixing between samples. Once all slots were filled, they were sealed with warm petrolatum jelly to prevent leaks around the slots.

Vertical electrophoresis was carried out at 80 mA for 5 hours using a Research Specialties power supply (max voltage 750, max. Amperage 200 mAmp). Pyronin Y dye (200 mg/100 ml) was used as the tracking dye. Gels were run also at 300 volts (40 mA) for 16 hours in order to get more resolution of the slow moving protein bands.

Detection of the proteins:

Upon completion of electrophoresis the gel was cooled in a refrigerator at 4-6°C for 30 minutes. After cooling, the gel was slabbed into 2 layers which were separated carefully and transferred to staining trays which had been filled previously with a thin layer of the dye solution to prevent gel adhesion and to ensure homogenous staining.

Some proteins stain better with one dye than other, therefore several dyes and combinations were used (Table II). After destaining, the R_f of the various protein components were compared by visual examination and plotted on charts. The distance migrated for each component was measured from the origin (sample slot) to both borders of each band. Protein patterns in each case were ascertained for at least three repetitions and the parental material was always included in each gel.

TABLE II

STAIN	STAINING TIME (hours)	DESTAINING AGENT	DESTAINING TIME (hours)
Nigrosine (0.025% in 10% acetic acid)	16	MeOH; HAc:H_2O 4: 1: 5	3 to 4
Amido Black 0.1% aqueous	6	distilled water	12
Amido Black-Nigrosine 0.5% in 5% HAc 0.025% in 10% HAc	10	Acetic acid	12
Nigrosine 0.1% aqueous	6	distilled water	24

TABLE II: Staining conditions.

RESULTS AND DISCUSSION

Until recently the most commonly used markers in evolutionary, taxonomic, and breeding experiments were morphological and cytological traits. Electrophoresis has more efficiency and resolving power, as a molecular technique, to identify and evaluate grain varieties and to differentiate between species (46,103,104,105,106). The main problem in the use of starch gel electrophoresis has been reproducibility of the protein patterns. This problem was minimized in our research by strict adherence to careful laboratory practices in the preparation of the gel. Thus, buffer, pH, time, temperature of starch heating, and gel pouring temperature, were kept constant.

After the conditions were standardized, the position and intensity of the bands were relatively constant. Therefore, because the conditions are standardized and gliadin synthesis is independent of environmental influences, the characteristic patterns obtained show primarily genotypic differences (45,46).

Extracted samples were stored frozen for long periods of time. Periodic analyses by starch gel electrophoresis were made to compare protein patterns. The number and position of proteins detected did not change with storage.

The proteins bands are reported diagramatically for better and more convenient evaluations. A key to the relative intensities is given.

Extraction of grain proteins

Even though the Osborne method has been the classical criteria to classify proteins, it is certain that the fractions extracted are not pure. An aqueous extract contains a representative profile of protein components and gives similar information as those of extracts with ethanolic or saline solution. Ethanol extracts also albumins and

globulins besides prolamines (46,47); prolamines corresponding to the fraction with low electrophoretic mobility (47).

An electropherogram of wheat proteins extracted with different solvents, in general, reveals three group of bands: a fast moving group, an intermediate group, and a slow moving group of proteins (Figure 2). Urea 2M gave a better extraction and better resolution of the components than the rest of the solvents used, although 2M urea also extracted some water soluble proteins (fast moving bands). In future discussions when we make reference to gliadins, or prolamines in general, we will be talking about proteins in the region of low electrophoretic mobility. This is based on work reported by Wrigley and Shepherd (105), Wrigley and McCausland (102) and others. Glutelin corresponds to the fraction remaining at the origin and was not present in the urea extract.

The same experiment was repeated for a variety of barley (Figure 3) Alcohol appears to extract larger amounts of one slow moving protein from barley than does aluminum lactate or urea, but does not extract as many different proteins. Hordein also shows sharper resolution in the ethanolic extract, but has only one band in the slow moving protein region. Rye was not studied in this way, due to a shortage of the parental seed. We decided to work under similar conditions for all the samples and chose 2M urea solution as the solvent to be used in all subsequent experiments.

Working with 2M urea we detected approximately 14 bands in wheat extracts, 12 in barley extracts, and 16 in rye extracts. The intensity of the bands varied suggesting different concentrations for each band detected.

Electrophoretic characteristics of the parental material:

Figure 4 shows the four varieties of barley under study. Within each variety the protein pattern is consistent but between varieties there are

Figure 2: Electropherograms of wheat proteins (Triticum durum var. Cocorit 71).

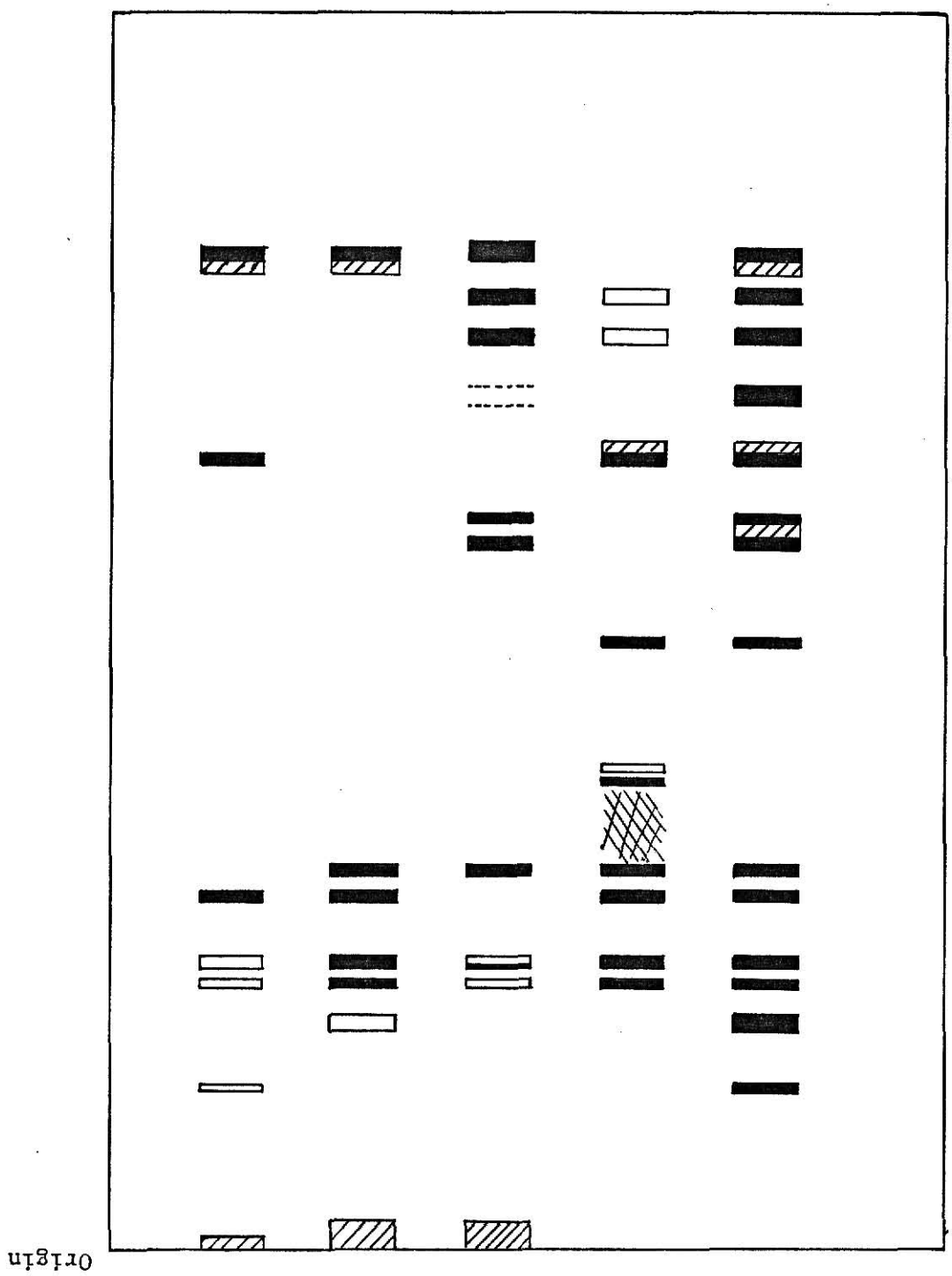

Figure 2: Wheat proteins.

Figure 3: Electropherograms of barley proteins (Hordeum vulgare var. Promesa).

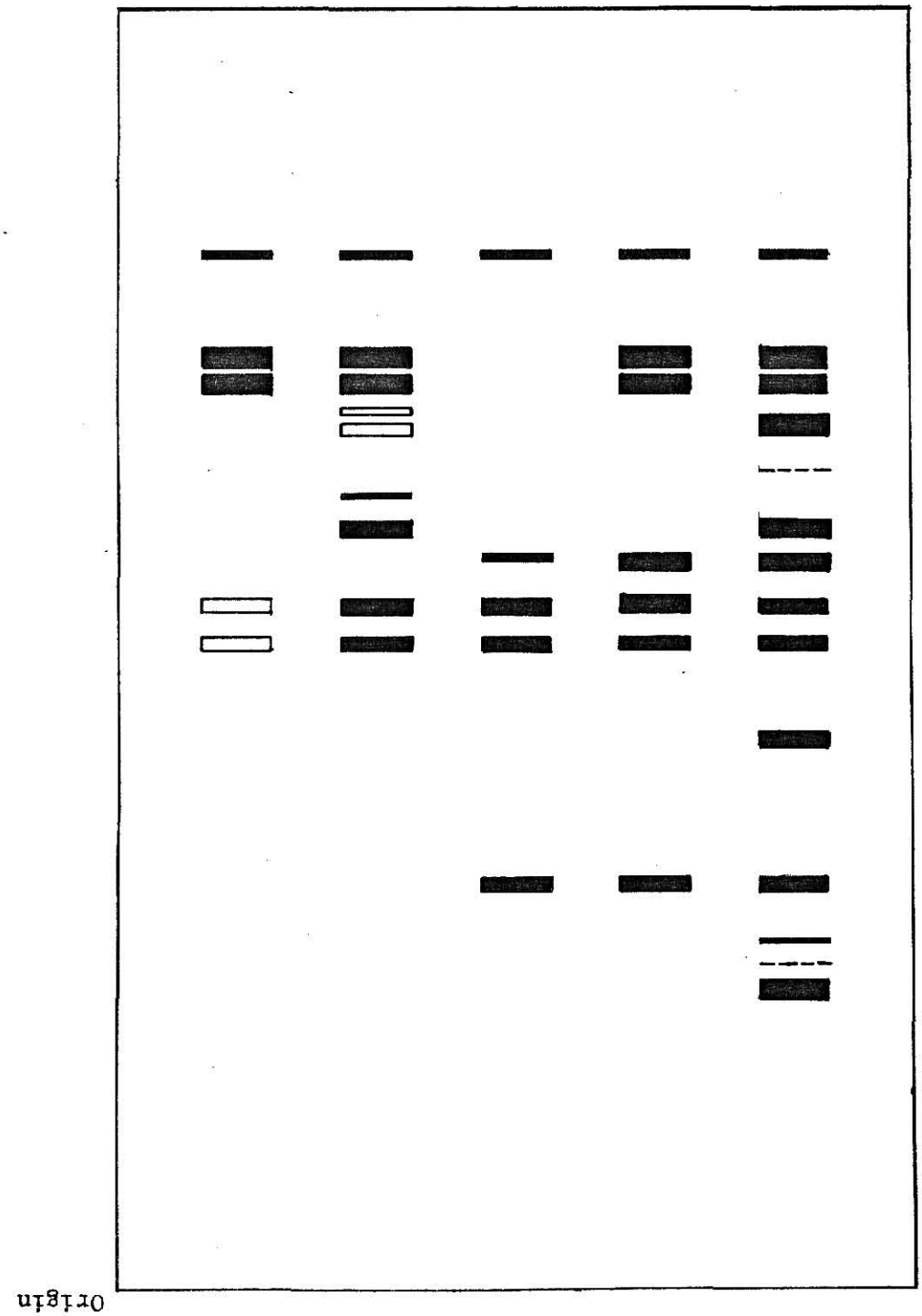

Figure 3: Barley proteins.

Figure 4: Electrophoretic characteristics of the parental material (Hordeum vulgare vars. Promesa, Porvenir, Apizaco, and CM67; Triticum aestivum var. Tobari 66; Triticum durum var. Cocorit 71 and Secale cereale var. Snoopy).

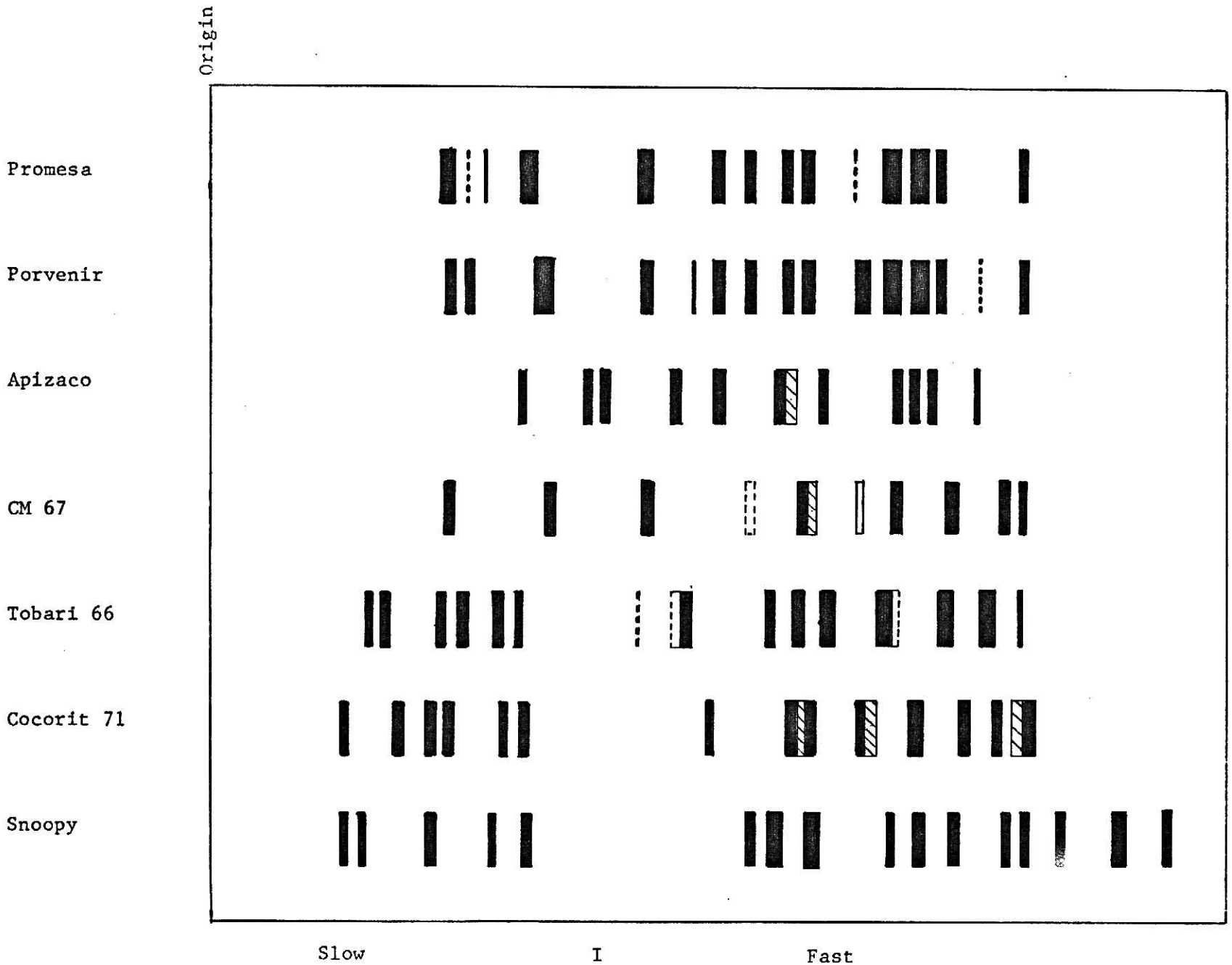

Figure 4: Parental material.

differences in number and position of the bands, and in staining intensity, which lets one recognize each variety on the basis of protein electrophoretic mobility and concentration. There are some common bands between varieties. For example, Promesa and Porvenir have similar patterns but there are certain bands which characterize the varieties. More common bands are observed in the fast moving region than in the slow moving region. Barley presents fewer slow moving bands than wheat or rye. The number of rye bands are similar to wheat. The prolamines of barley are much less soluble than those of wheat and rye. These results coincide with those reported by Elton and Ewart (52) and Coulson and Sim (47).

Comparison between electropherograms of hybrids and their corresponding parental species:

Starch gel electrophoresis has been helpful to obtain information about relationships between Triticineae species. Most of this information has been used to establish protein synthesis pathways, to identify the evolutionary relationships of the tribe Triticineae by genome characterization, and to differentiate parents from hybrids by their protein components. Attempts to study relationships between hybrids and their parents was first reported by Moritz (107) who found that the antigen composition of Triticale endosperm was the sume of the composition of the two parents. Hall (37) used immunoelectrophoresis to find that "all the demonstrable proteins of wheat were present in Triticale, but some rye proteins were not found back". Hall also detected one protein component in the rye-wheat extract which was not present in either of the two parent species. Later on, and with aid of electrophoresis, the additive nature of the protein pattern represented in the hybrid was reported (34,35,39,108). Johnson (36) concluded that "the electrophoretic pattern

of one synthetic amphiploid may be predicted from the paternal parents or in other words the protein involved in a cross are conservative enough to maintain their essential homologies in the course of evolution".

The phenomena of "new hybrid protein" in triticale is somewhat different than ours because in triticale there is one genome from rye present, whereas the foreign genome has been eliminated in our hybrids. Only a limited number of genes are believed to have been transferred (2,3).

On the basis to those previous experiments it was attempted to study gliadins and other proteins extracted with 2M urea as a measure of gene transfer between wheat, barley, and rye.

In order to facilitate the description of changes occurring in the protein electrophoretic patterns of hybrids, with respect to the maternal parents, the pattern was divided into 3 zones: slow, intermediate and fast moving bands, and the bands have been numbered in each zone. This classification is arbitrary and only represents an attempt to clarify which bands are missing and which are additional.

Figures 5,6,7 and 8 and Table 3 show the electrophoretic characteristics of the protein patterns obtained. In general, the progenies presented patterns similar to their maternal parents as was expected. However some interesting anomalies deserve attention:

1. Some bands present in the maternal parent were absent in the protein pattern of the progeny.
2. Some protein bands present in the progeny were absent in the maternal parent, but similar protein bands were present in the paternal parent and,
3. Some protein bands in the progeny were not present in either of the parents.

Figure 5: Electropherograms of hybrid progenies and their corresponding parents (Triticum aestivum var. Tobari 66 x Hordeum vulgare vars. Promesa and Porvenir).

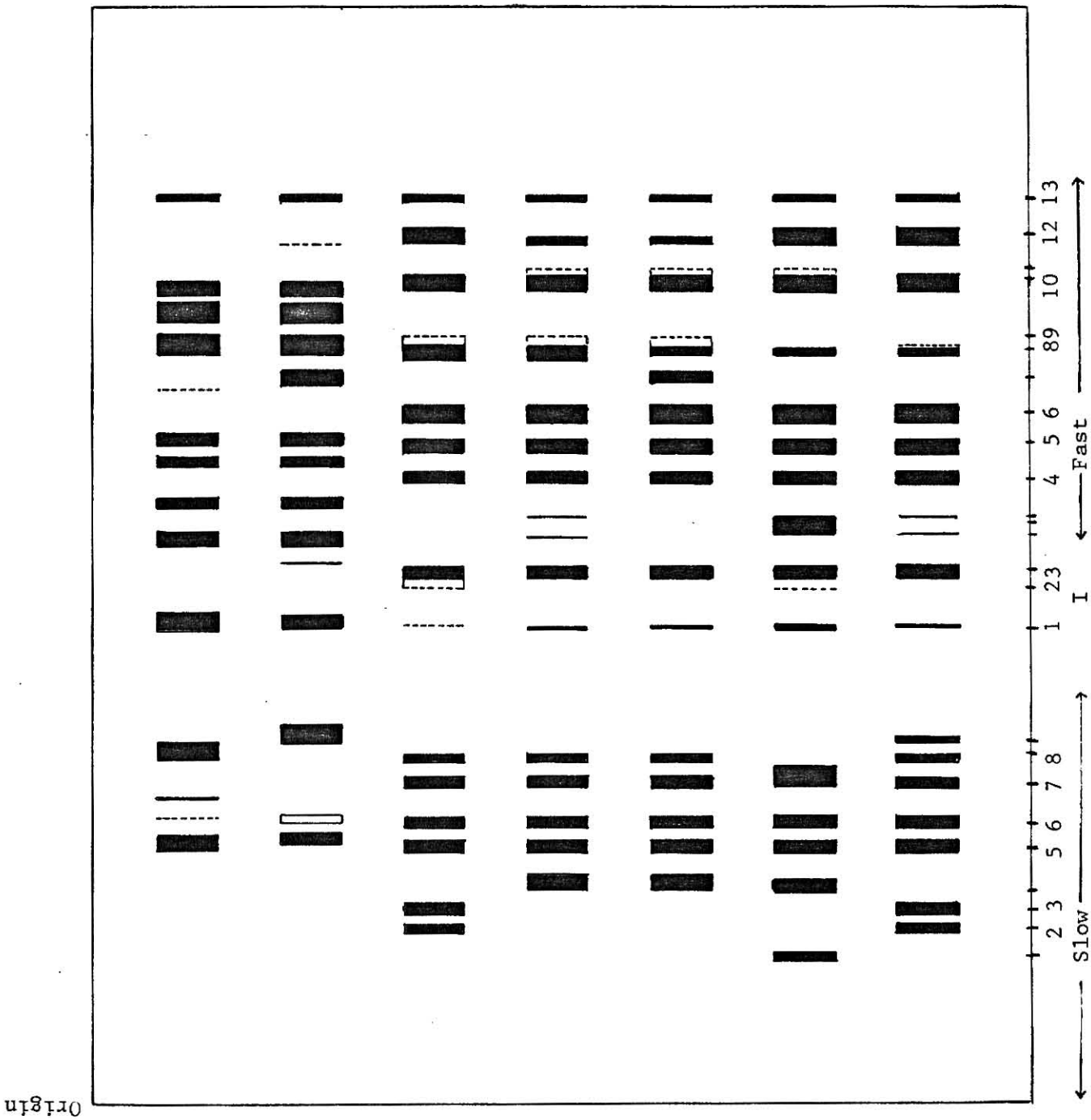

Figure 5: Triticum aestivum x Hordeum vulgare

Figure 6: Electropherograms of hybrid progenies and their corresponding parents (Triticum durum var. Cocorit 71 x Hordeum vulgare var. Promesa).

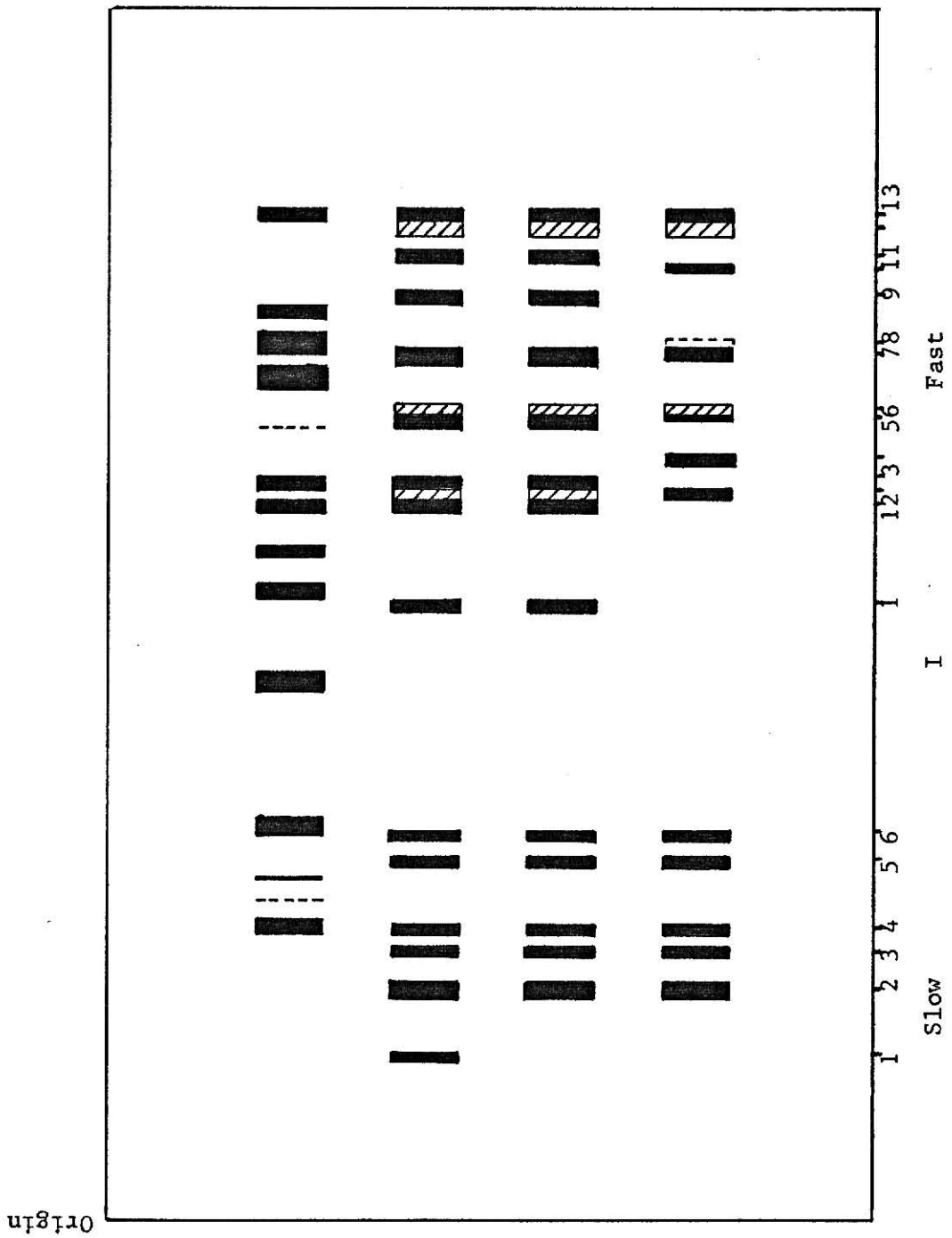

Figure 6: Triticum durum x Hordeum vulgare

Figure 7: Electropherograms of hybrid progenies and their corresponding parents (Triticum durum var. Cocorit 71 x Hordeum vulgare var. Apizaco).

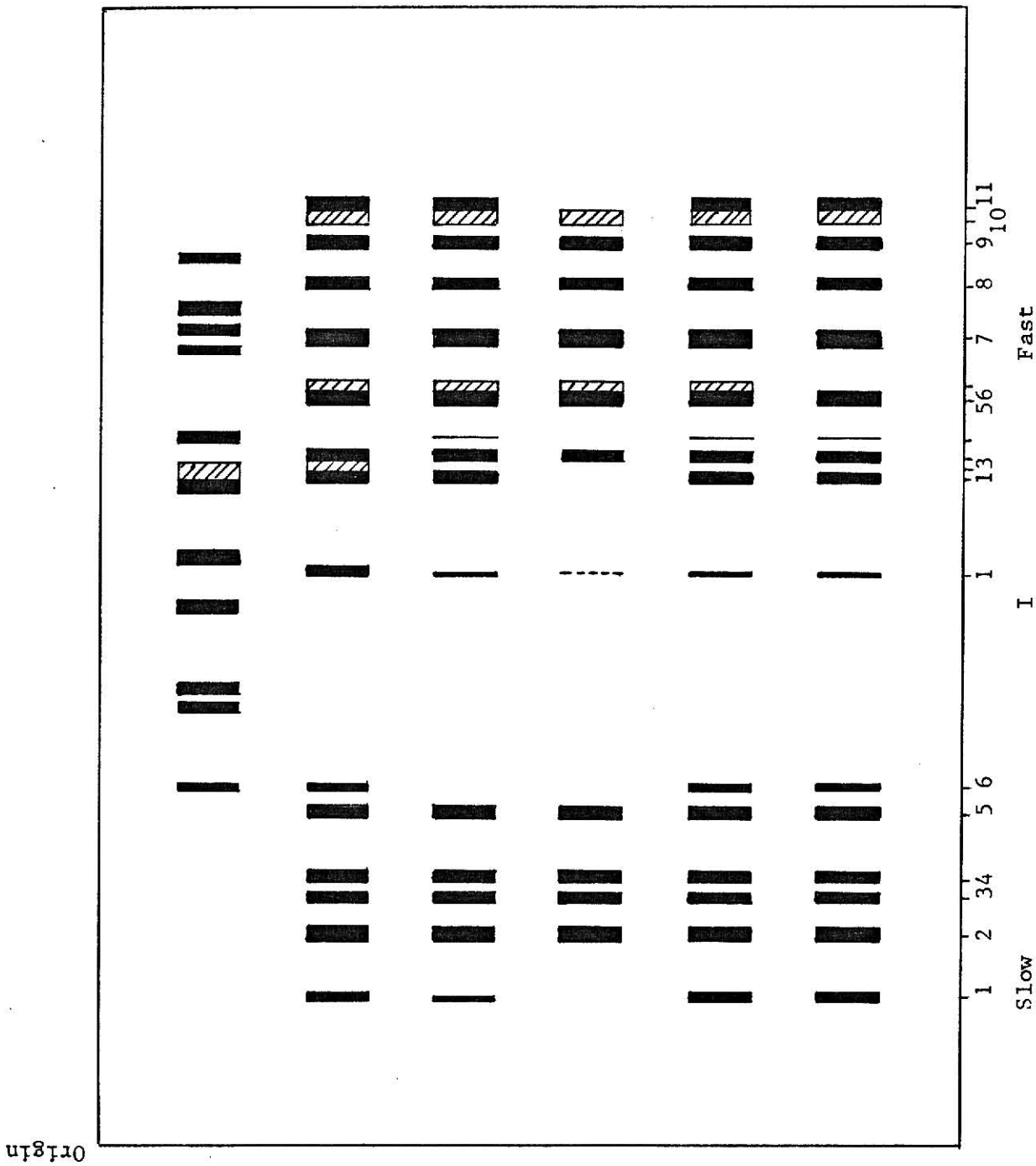

Figure 7: Triticum durum x Hordeum vulgare.

Figure 8: Electropherograms of hybrid progenies and their corresponding parents (Secale cereale var. Snoopy x Hordeum vulgare var. CM67).

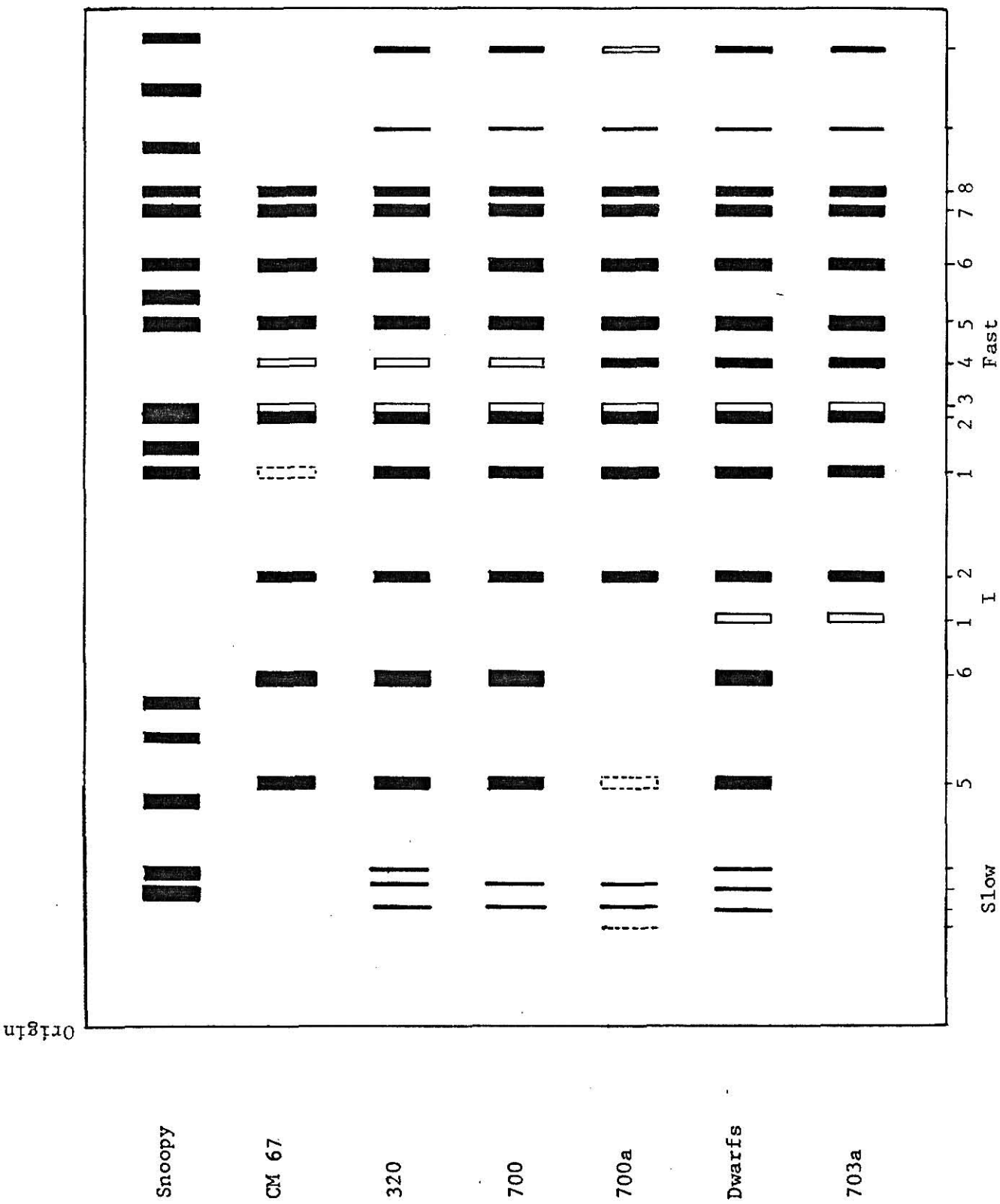

Figure 8: Secale cereale x Hordeum vulgare.

TABLE NO. 3

Distinctive characteristics of electrophoretic patterns of hybrid protein.

Hybrid Material	Slow moving proteins			Intermediate			Fast moving proteins		
	added	deleted	common	added	deleted	common	added	deleted	common
Tob x Por 693	4	2,3	5,6,7,8	-	2	1,3	1,3,11	(12)	4,5,6,8,9,10,12,13
Tob x Por 693a	4	2,3	5,6,7,8	-	2	1,3	7	(12)	4,5,6,8,9,10,12,13
Tob x Por 54c	9	-	2,3,5,6,7,8	-	2	1,3	-	(9), 11	4,5,6,8,9,10,12,13
Tob x Prom 665	1,4,7	2,3	5,6	-	-	1,2,3	-	-	4,5,6,8,9,10,12,13
Coc x Prom 691 c & f	-	1	2,3,4,5,6	-	-	1	-	-	1,2,3,5,6,7,9,11, 12,13
Coc x Prom 691d	-	1	2,3,4,5,6	-	1	-	4,8,10	1,3,9,11	2,5,6,7,12,13
Coc x Apiz 675a	-	6	1,2,3,4,5	-	-	1	4	2	1,3,5,6,7,8,9,10,11
Coc x Apiz 675b	-	1,6	2,3,4,5	-	-	1	-	1,2,11	3,5,6,7,8,9,10
Coc x Apiz 666	-	-	1,2,3,4,5,6	-	-	1	4	2	1,3,5,6,7,8,9,10,11
Coc x Apiz 679	-	-	1,2,3,4,5,6	-	-	1	4	2,6	1,3,5,7,8,9,10,11
CM67 x Sno. 320	2,3,4	-	5,6	-	-	2	9,10		1,2,3,4,5,6,7,8
" 700	2,3	-	5,6	-	-	2	9,10		1,2,3,4,5,6,7,8
" 700a	1,3	5,6	5	-	-	2	9,10		1,2,3,4,5,6,7,8
" Dwarfs	2,3,4	-	5,6	1	-	2	9,10		1,2,3,4,5,6,7,8
" 703a	-	5,6	-	1	-	2	9,10		1,2,3,4,5,6,7,8

Thus, after the fifth generation the hybrid progeny has the same morphological and cytogenetical characteristics as the maternal parent (except they have segregated for some special traits such as height and disease reaction) but with a novel electrophoretic pattern. Bates, et al. (98,99) gave some indications about how the introgression has taken place, explaining the phenomena on the basis of the chemicals used. We know that gene function is controlled by different mechanisms, and there exist the possibility of transferring a gene which turns off the synthesis of a particular protein. It is also possible that some fragments of DNA from the paternal parent were inserted into the DNA of the maternal parent before the elimination of the paternal chromosomes (109,110).

Some differential and unidirectional transfer and insertion of polynucleotides from paternal DNA to the maternal genome could be expected in cases where the genomes have a high DNA sequence homology. That depends on how closely the species are related and it is known already that some homology exists between rye, wheat, and barley (84).

It is suspected that the chemicals used might affect the attachment of DNA to the nuclear membrane (3). According to Bates, et al. (3) paternal DNA with high sequence homology "may be competing for common membrane attachment points and upsetting replication". Excission - repair mechanisms are involved in this process including complex enzymatic reactions (79); thus, fragmentation and insertion of foreign DNA might happen. DNA replication could occur with the new sequence while the rest of the DNA is eliminated (109,110).

This type of insertion is similar to the one presented for virus and bacteria (111,112) resulting in insertion of paternal chromosomes into the maternal genome without modification of the maternal phenotype.

Of course if there is any alternation of the amino acid sequence, the net charge, molecular weight and the molecular shape of the protein probably will be changed. Thus, the total effect of the changes should be reflected in protein mobility in electrophoresis.

Long electrophoresis time can resolve more components for the region of low electrophoretic mobility into clearly defined bands. Figures 9 and 10 show a more detailed resolution of the proteins illustrated before in electropherograms 5 and 6 due to the longer separation time (16 hours) at a low voltage (130 volts, 40 mAmp.). Table 3 gives the distinctive features of the slow moving gliadin bands. Gliadins evidently are composed of a large number of components. Thus, bands which appeared as one in Figure 5 were resolved into a great number of components. This technique reveals striking differences between the hybrid material and the maternal parents. The new protein patterns given evidence that some barley or rye DNA was transferred and expressed in the proteins separated by starch gel electrophoresis.

Figure 9: Protein electrophoretic patterns of hybrid progenies and their corresponding parents performed at 40 mAmp for 16 hours. (Triticum aestivum var Tobari 66 and Hordeum vulgare vars. Promesa and Porvenir).

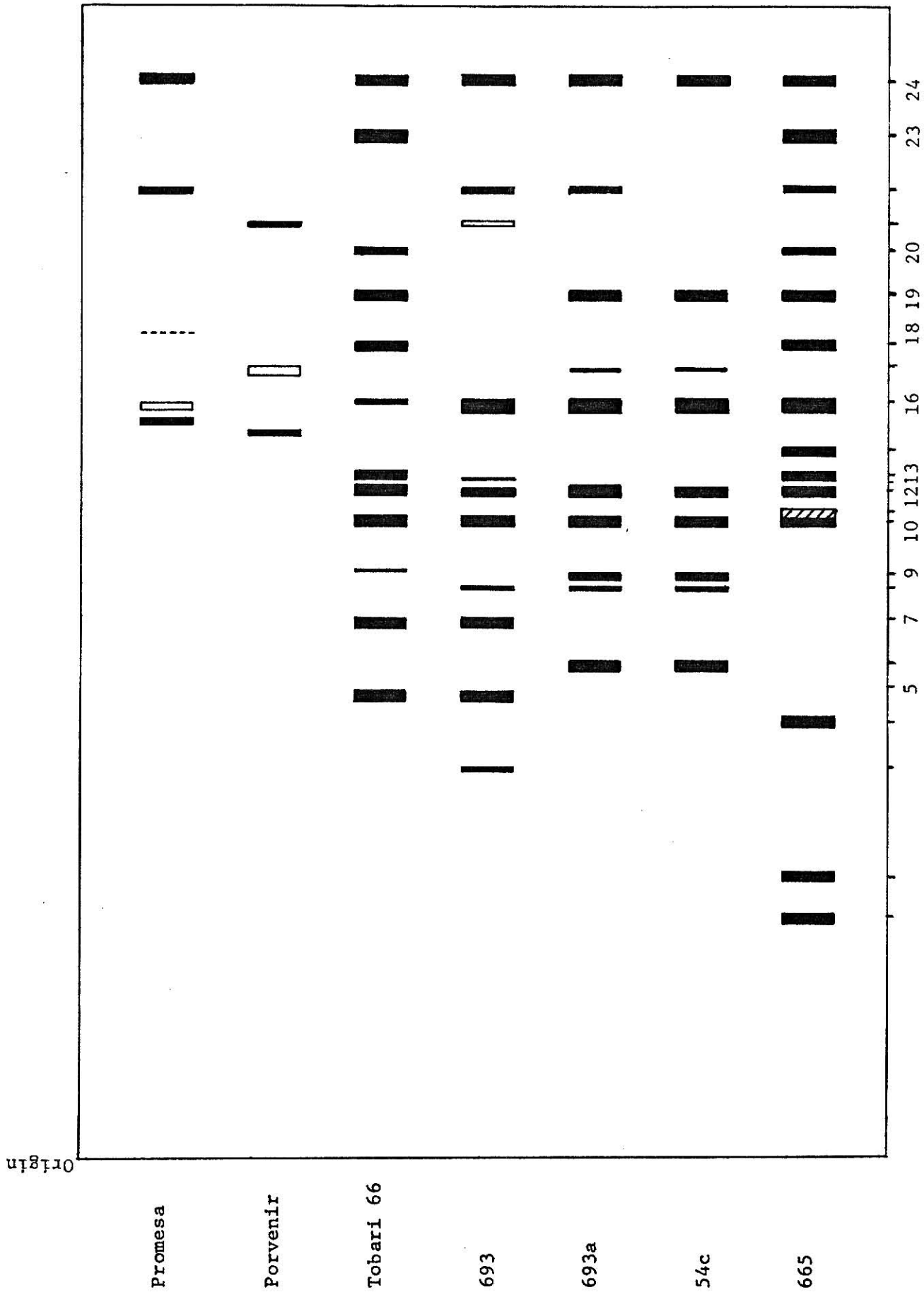

Figure 9: Triticum aestivum x Hordeum vulgare

Figure 10: Protein electrophoretic patterns of hybrid progenies and their corresponding parents performed at 40 mAmp for 16 hours (Triticum durum var. Cocorit 71 x Hordeum vulgare var. Promesa).

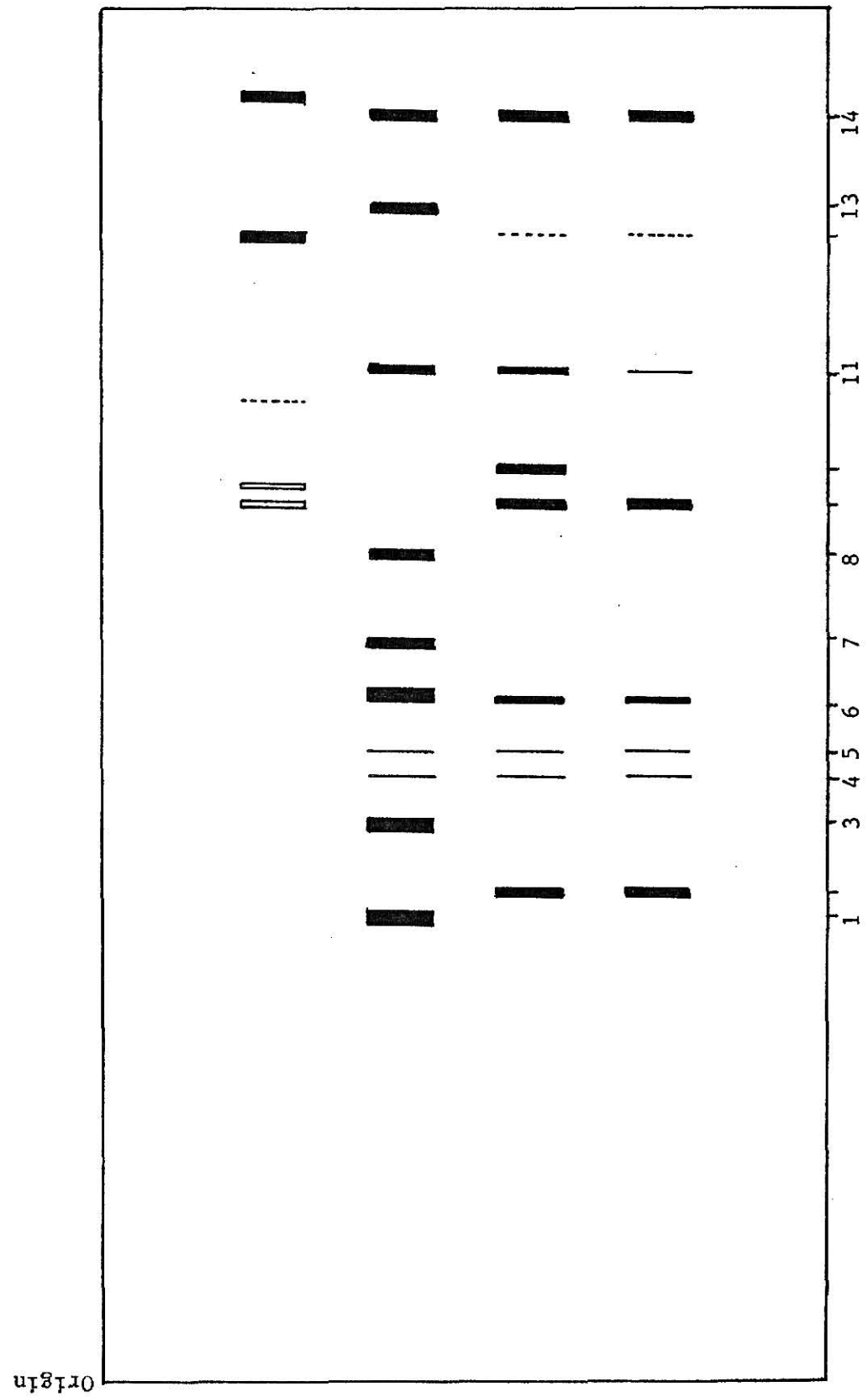

Figure 10: Triticum durum x Hordeum vulgare.

TABLE No. 4

Distinctive characteristics of slow moving protein bands in hybrids, as compared with their respective maternal parents.

Material	Bands added	Bands deleted	Common bands
Tobari x Porvenir			
693	3,8,21,22	10,18,19,20,23	5,7,11,13,14,16,24
693a	6,8,9,17,22	5,7,10,14,18,20,23	11,13,16,19,24
54c	6,8,9,17	5,7,10,14,18,20,23	11,13,16,19,24
Tobari x Promesa			
665	1,2,4,12,15,22	5,7,10	11,13,14,16,18,19,20 21,23
Cocorit x Promesa			
691 c and f	2,9,10,12	1,3,7,8,13	4,5,6,11,14
691d	2,9,12	1,3,7,8,13	4,5,6,11,14

LITERATURE CITED

1. FAO, Food Balance Sheets, Rome (1970).
2. BATES, L.S., CAMPOS, A., RODRIQUEZ, R. and ANDERSON, R.G., Progress toward new cereals. Cereal Sci. Today. 19: 283 (1974).
3. BATES, L.S., MUJEEB, K.A. and WATERS, R.F. Wheat and Barley Hybrids. Problems and Potentials. Cereal Research Communications., 4: 377 (1976).
4. OSBORNE, T.B., and VOORHEES, C.G. The protids of the wheat kernel. Amer. Chem. J. XV: 392 (1893).
5. MARES, D.J., NORSTOG, K. and STONE, B.A. Early stages in the development of wheat endosperm. I. The change from free nuclear to cellular endosperm. Aust. J. Bot. 23: 311 (1975).
6. GRAHAM, J.S.D., JENNINGS, A.C., MORTON, R.K., PALK, B.A. and RAISON, J.K. Protein bodies and protein synthesis in developing wheat endosperm. Nature 196: 967 (1962).
7. WRIGLEY, C.W. and SHEPERD, K.W. Electrofocusing of grain proteins from wheat genotypes. Ann. N.Y. Acad. Sci. 209: 154 (1973).
8. MOSSE, J. Alcohol-soluble proteins of cereal grains. Federation Proceedings, 25: 1663 (1966).
9. WU, Y.V. and DIMLER, R.J. Hydrogen ion equilibria of wheat gluten. Arch. Biochem. Biophys. 102: 230 (1963).
10. WU, Y.V. and DIMLER, R.M. Hydrogen ion equilibria of wheat gluten and gliadin. Arch. Biochem. Biophys. 103: 310 (1963).
11. HOLME, J. and BRIGGS, D.R. Studies on the physical nature of gliadin. Cereal Chem. 36: 321 (1959).
12. BROHULT, S., SANDEGREN, E., NEUTRATH, H., and BAILEY, K. The proteins Vol. II. Academic Press, Inc., New York, p. 487 (1954).

13. OSBORNE, T.B. The vegetable protein. 2nd ed. Longmans, Green and Co., London. p. 25 (1924).

14. McCALLA, A.G. and GRALEN, N. Ultracentrifugue and diffusion studies of gluten. Can. J. Research 20, Sec. C., 130 (1942).

15. VERCOUTEREN, R. and LONTIE, R. The solubilization of wheat gluten by dimethyl formamide. Arch. Intern. Physiol. 62: 579 (1954), CA-52: 1309.

16. MINETTI, M., PETRUCCI, T., PECHIARI, F., SILANO, V., and AVELLA, R. Varietal differences in water-soluble gliadin fractions of Triticum aestivum and of Triticum durum seeds. J. Sci. Food Agric. 22: 72 (1971).

17. WOYCHICK, J.H., BOUNDY, J.A. and DIMLER, R.J. Starch gel electrophoresis of wheat gluten proteins with concentrated urea. Arch. Biochem. Biophys. 94: 477 (1961).

18. JONES, R.W., TAYLOR, N.W. and SENTI, F.R. Electrophoresis and fractionation of wheat gluten fractions. Arch. Biochem. Biophys. 94: 363 (1959).

19. ELTON, G.A.H. and EWART, J.A.D. Starch gel electrophoresis of wheat proteins. Nature (London) 187: 600 (1960).

20. COULSON, C.B. and SIM, A.K. Starch gel electrophoresis of isolated wheat gluten. Biochem. J. 80: 46P (1961).

21. GRAHAM, J.S.D. Starch gel electrophoresis of wheat flour proteins. Aust. J. Biol. Sci. 16: 342 (1963).

22. HEUBNER, F.R. and WALL, J.S. Improved chromatographic separation of gliadin proteins of ion exchange cellulose. Cereal Sci. Today. 10: 166 (1965).

23. SCHWERT, G.W., PUTNAM, F.W. and BRIGGS, D.R. An electrophoretic study of gliadin. Arch. Biochem. 4: 371 (1944).

24. TAYLOR, N.W. and CLUSKEY, J.E. Gluten and its glutenin component: viscosity, diffusion and sedimentation studies. Arch. Biochem. Biophys. 97: 399 (1962).

25. CLUSKEY, J.E. and WU, Y.V. Optical rotatory dispersion of wheat gluten, gliadin, and glutenin in acetic acid and aluminum lactate systems. Cereal Chem. 43: 119 (1966).

26. WU, Y.V., CLUSKEY, J. and SEXSON, K.R. Effect of ionic strength on the molecular weight and conformation of wheat gluten proteins in 3M urea solutions. Biochim. Biophys. Acta. 133: 83 (1967).

27. COULSON, C.B. and SIM, A.K. Wheat proteins. I. Fractionation and varietal variation of endosperm proteins of Triticum vulgare. J. Sci. Food Agric. 16: 458 (1965).

28. COLE, E. and MECHAM, D.K. Cyanate formation and electrophoretic bahavior in gels containing urea. Anal. Biochem. 14: 215 (1966).

29. WU, Y.V., and DIMLER, R.J. Conformational studies of wheat gluten, glutenin, and gliadin in urea solution at various pH's. Arch. Biochem. Biophys. 107: 435 (1964).

30. LEE, J.W. Preparation of gliadin by urea extraction. J. Sci. Food Agric. 19: 153 (1968).

31. BARBER, N.H., DRISCOLL, C.J., LONG, P.M. and VICKERY, R.S. Protein genetics of wheat and homoelogous relationships of chromosomes. Nature, 218: 450 (1968).

32. INGRAM, V.M. Abnormal human haemoglobin I -- the comparison of normal human and single cell haemoglobin by fingerprinting. Biochim. Biophys. Acta 28: 539 (1958).

33. RILEY, R. and KIMBER, G. The transfer of alien genetic variation to wheat. Rep. Pl. Breed. Inst. 1964-65:33 (1966).

34. JOHNSON, L.B. and HALL, O. Analysis of phylogenetic acitivities in the tritinaceae by protein electrophoresis. Am. J. Bot. 52: 506 (1965).

35. JOHNSON,L .B., BARNHART, D. and HALL, O. Analysis of genome and species relationship in the polyploid wheats by protein electrophoresis. Am. J. Bot. 54: 1089 (1967).

36. JOHNSON, L.B. The protein electrophoresis approach to species relationship in wheat. In. R. Bogart, Genetic Lectures. Vol. 1 page 19. Oregon State University Press, Oregon (1969).

37. HALL, O. Immunoelectrophoretic analysis of allopolyploid rye-wheat and its parental species. Hereditas 45: 495 (1959).

38. CHEN, C.J. and BUSHUK, W. Nature of proteins in triticale and its parental species. Can. J. Plant Sci. 50: 25 (1970).

39. HALL, O. and JOHNSON, L.B. Electrophoretic analysis of the amphiploid Stipa viridula and Oryzopsis hymenoides and its parental species. Hereditas 48: 530 (1963).

40. BOYD, W.J.R., LEE, J.W. and WRIGLEY, C.W. The genome and the control of wheat gluten synthesis. Experientia. 25: 317 (1969).

41. BARBER, H.N., DRISCOLL, C.J. and VICKERY, R.S. Enzymic markers for wheat and rye chromosomes. In: "Third International Wheat Genetics Symposium". Ed. by Finlay, K.W. and Shepherd, K.W., Australian Academy of Science, Canberra, Australia. 116 (1968).

42. YONG, F.C. and UNRAU, A.M. Influence of alien genome combinations on protein synthesis in cereals. Can. J. of Biochem. 42: 1647 (1964).

43. SCHWARTZ, D., TUCHSMAN, L. and McGRATH, K.H. Allelic isozymes of the pH 7.5 esterase in maize. Genetics 52: 1265 (1964).

44. KRUSE, A. Intergeneric hybrids between Triticum aestivum L. (V. Koga II, 2n=42) and Avena sativa L. (var. Stal, 2n=42) with pseudogamous seed formation. Preliminary report in Royal Veterinary and Agriculture College Yearbook, page 188, Copenhagen, Denmark (1969).

45. LEE, J.W. and RONALDS, J.A. Effect of environment on wheat gliadin. Nature, 213: 844 (1967).

46. DOEKES, G.J. Wheat grain proteins. Analysis of varieties by starch gel electrophoresis. Thesis, University of Leiden (1969).

47. COULSON, C.B. and SIM, A.K. Proteins of various species of wheat and closely related genera and their relationship to genetical characteristics. Nature. 202: 1305 (1964).

48. WRIGLEY, C.W. Protein mapping by combined gel electrofocusing and electrophoresis: Application to the study of genotypic variations in wheat gliadins. Biochem. Genetics. 4: 509 (1970).

49. MESROB, B. and IVANOV, C. Comparative fractional composition of hordein from different barley varieties. Dokl. Bolg. Akad. Navk. 28: 291 (1970). C.A.: 73:73812h.

50. WALDSCHMIDT-LUTZ, E. Variations in the composition of nitrogen materials in barley. European Brewery Vonv., Proc. Congr. 7th Rome. page 37 (1959). C.A.: 54:21342a.

51. SHEPHERD, K.W. Chromosomal control of endosperm protein in wheat and rye. In: "Third International Wheat Genetics Symposium". Ed. by Finlay, K.W. and Shepherd, K.W. page 86. Australian Academy of Science. Canberra. Australia (1968).

52. BISERTE, G., SCRIBAN, R. The proteins of barley. Bull Soc. Chim. Biol. 32: 959 (1950). C.A.: 45:51959.

53. ELTON, G.A.H. and EWART, J.A.D. Electrophoretic comparison of cereal proteins. J. Sci. Food Agric. 15: 119 (1964).

54. EWART, J.A.D. Amino acid analysis of cereal flour proteins. J. Sci. Food Agric. 18: 548 (1967).

55. EWART, J.A.D. Fractional extraction of cereal flour proteins. J. Sci. Food Agric. 19: 241 (1968).

56. ENARI, T., MIKOLA, J. and NUMMI, M. Starch gel electrophoresis of basic water soluble barley grain proteins. Acta Chim. Scand., 16: 517 (1962).

57. IVANOV, C.H., MESROB, B. and PRUZIK, Z. Fractionation of Hordein by preparative continuous carrier free electrophoresis. Can. J. Biochem. 46: 1301 (1968).

58. MESROB, B., PETROVA, M. and IVANOV, CH.P. A comparative study of hordein fractions. Biochim. Biophys. Acta. 200: 459 (1970).

59. MESROB, H. and KANCHEVA, V. Analytical separation of hordein and its fractions by disc electrophoresis on acrylamide gel. J. Chromat. 46: 94 (1970).

60. BROWN, D. and BOYD, W.J.R. Separation of barley proteins by disc electrophoresis using polyacrylamide. J. Ins. Brew. 72: 541 (1966). CA: 6636589a

61. PERUANSKU, Y.V. and SADYKOV, S.S. Electrophoretic fractionation of barley hordein in polyacrylamide gel. Vestn. Sel' Skohoz. Nauki. 115: 37 (1972). C.A.: 78:81686.

62. DAFTARY, R.D. and POMERANZ, Y. Dispersability of cereal proteins in urea solution. J. Sci. Food. Agric. 17: 72 (1966).

63. EWART, J.A.D. Fingerprinting of glutenin and gliadin. J. Sci. Food. Agric. 17: 30 (1966).

64. MERTZ, E.T. Recent improvement in corn proteins. In: Symposium: Seed proteins. page 136 Inglett. G.E. Ed. AVI Pu. Co. Westport, Conn (1972).

65. MUNCK, L. Barley seed proteins. In: Symposium: Seed proteins, page 144. Inglett, G.E. Ed. AVI Pu. Co. Westport, Conn. (1972).

66. MUNCK, L. Improvement of nutritional value in cereals. Hereditas. 72: 1 (1972).

67. CLARK, W.L. and POTTER, G.C. The composition and nutritional properties of proteins in selected oat varieties. In: Symposium: Seed Proteins, page 165. Inglett, G.E. Ed. AVI Pu. Co. Westport, Conn. (1972).

68. MUNCK, L., KARLSON, K.E., HAGBERG, A. Selection and characterization of high protein high lysine variety from the world collection. In: Proceedings 2nd. International Conference Barley Genetics Symposium, Washington State Univ. Press, page 544 (1971).

69. SINGH, R. and AXTEL, L. High Lysine mutant gene (hl) that improves protein quality and biological value of grain sorghum. Crop Science 13: 535 (1973).

70. HISCKE, H.H., POTTER, G.C. and GRAHAM, W.R. Nutritive value of oat protein. I. Varietal differences as measured by amino acid analysis and rate growth response. Cereal Chem. 45: 374 (1968).

71. NELSON, O.E. The modification of mutation of protein quality of maize. Joint FAO/EAEA. Division of Atomic Energy in Food and Agriculture, New approaches to breeding for improved plant protein panel. Proc. Series. STI/PUB/212. Intern. Atomic Energy Agency. Vienna. 41 (1960).

72. RUCKMAN, J.E., ZCCHEILE, F.P., Jr., and QUALSET, C.O. Protein, lysine and grain yields of triticale and wheat as influenced by genotype and location. J. Agric. Food Chem. 21: 696 (1973).

73. LORENZ, K. Food uses of triticale: hybrid of wheat and rye. Food Tech. 11: 66 (1972).

74. JOHNSON, V.A., and LAY, C.L. Genetic improvement of plant proteins. J. Agric. Food Chem. 22: 558 (1974).

75. MERTZ, E.T. Potential for genetic improvement of plant protein quality. In: "Amino acid fortification of protein food". Ed. by Scrinishaw, N.S. and Altschul, A.M. Eds., MIT Press, Cambridge, Mass. p. 98 (1969).

76. NELSON, O.E. Genetic modification of protein quality in maize. Adv. Agron. 21: 171 (1969).

77. JOHNSON, V.A., MATTERN, P.J. and SCHMIDT, J.W. The breeding of wheat and maize with improved nutritional value. Proc. Nut. Sci. 29: 20 (1970).

78. CRICK, F.H.C., BARNETT, L., BRENNER, S. and WATTS-TOBIN, R.J. General nature of the genetic code for protein. Nature 192: 1227 (1961).

79. ELKIND, M.M., and KAMPER, C. Two forms of repair of DNA in mammalian cells following irradiation. Biophys. Journal, 10: 237 (1970).

80. ADAMS, M. The need for rapid assay of protein quality. J. Agric. Food Chem. 22: 556 (1974).

81. WRIGLEY, C.W. Biochemistry of the wheat protein complex and genetic control. Cereal Science Today. 17: 370 (1972).

82. ORTH, R.A. and BUSHUK, W. Studies of glutenin. VI. Chromosomal location of genes coding for subunits of glutenin of common wheat. Cereal Chem. 51: 118 (1974).

83. BELL, G.D.H. The comparative phylogenia of the temperate cereals In: Essays on crop plant evolution. Ed. by J. Hutchinson. Cambridge Univ. Press. London (1965).

84. BENDICH, A.J. and McCARTHY, B.J. DNA Comparisons among barley, oats, rye and wheat. Genetics 65: 545 (1970).

85. BRINK, R.A., COOPER, D.C. and AUSHERMAN, L.E. A hybrid between Hordeum jubatum and Secale cereale. J. Heredity, 35, 67 (1944).

86. GORDON, G.S. and RAW, A.R. Wheat-barley matings. J. Agric. (Victoria, Australia) 30: 138 (1932).

87. KARPECHENKO, G.B. Tetraploid barleys obtained by high temperature treatments. Biol. Zhurnal, 7: 287 (1938).

88. THOMPSON, W.P. The frequency of fertilization and the nature of embryo and endosperm development in intergeneric crosses in cereals. VII Int. Congress Genet. Proc. (Abstract).

89. QUINCKE, F.L. Interspecific and intergeneric crosses with Hordeum Can. J. Res. C.18: 372 (1940).

90. SMITH, D.C. Intergeneric hybridization of cereals and other grasses. J. Agric. Res. 64: 3347 (1942).

91. SMITH, D.C. Intergeneric hybridization of Triticum and other grasses principally Agropyron. J. Hered 34: 219 (1943).

92. THOMPSON, W.P. and JOHNSTON, D. The cause of incompatibility between barley and rye. Can. J. Res. 23: 1 (1945).

93. SICLOVAN. V. A new method for obtaining hybrid grains between barley and wheat. Plant Breeding Abstract 26: 575 (1956).

94. KRUSE, A. Intergeneric hybrids between Hordeum vulgare L. ssp. distichum (v. Pallas 2n=14) and Secale cereale L. (v. Petkus, 2n=14). Royal Vet. and Agric. College Yearbook, page 82, Copenhagen, Denmark (1967).

95. HOD'KOV, L.E. Distant hybridization of barley. Plant Breeding Abs. 40: 657 (1970).

96. KRUSE, A. Hordeum vulgare ssp. disticum (var. Bomi) x Triticum aestivum (var. Koga). An F_1 hybrid with generative seed formation. Hereditas, 78: 319 (1974).

97. KIMBER, G. and SALLEE, P.J. A hybrid between Triticum timopheevi and Hordeum bodganii. Cereal Res. Comm. 4: 33 (1976).

98. BATES, L.S., RODRIGUEZ, R., WATERS, R.F. and MUJEEB, K.A. Wide hybridization: gene transfer from barley and rye. Annual Wheat Newsletter, 22: 84 (1976).

99. BATES, L.S., MUJEEB, K.A., RODRIGUEZ, R., WATERS, R.F. Rye dwarf gene introgression into barley. Barley Genetics Newsletter 6: 7 (1976).

100. BATES. L.S. Future grains. Cereal Foods World. 20: 544 (1975).

101. BATES, L.S. and DEYOE, C.W. Wide hybridization and cereal improvement. Economic Botany. 27: 401 (1973).

102. WRIGLEY, C.W. and McCAUSLAND, J. Instruction manual on laboratory procedures for identifying grain samples of Australian wheat varieties. C.S.I.R.O. Wheat Research Unit. Technical Publication No. 3. (1975).

103. ALMGARD, G. and NORMAN, T. Biochemical Technique as an aid to distinguish some cultivars of barley and oats. Agric. Hortique. Genetica. 28: 117 (1970).

104. ELLIS, R.P. The identification of wheat varieties by the electrophoresis of grain proteins. The Journal of the National Institute of Agriculture Botany. 12: 223 (1971).

105. WRIGLEY, C.W. and SHEPPERD, K.W. Identification of Australian wheat cultivars by laboratory precedures: examination of pure samples of grain. Australian Journal of Experimental Agriculture and Animal Husbandry. 14: 796 (1974).

106. WRIGLEY, C.W. and BAXTER, R.I. Identification of Australian wheat cultivars by laboratory procedures: grain samples containing a mixture of cultivars. Australian Journal of Experimental Agriculture and Animal Husbandry. 14:805 (1974).

107. MORITZ, C.A. Serologische Untersuchungen and Getreidebastarden. Ber. deut. botan. Ges. 51: 52 (1933).

108. JOHNSON, L.B. Confirmation of the genome donor of Aegilops cylindrica Nature. 26: 859 (1967).

109. SUBRAHMANYAM, N.C. and KASHA, K.J. Selective chromosomal elimination during haploid formation in barley following interspecific hybridization. Chromosoma 42: 111 (1973).

110. BARCLAY, J.R. High frequencies of haploid production in wheat (Triticum aestivum) by chromosome elimination. Nature 256: 410 (1975).

111. LANDY, A., FOELLER, C., and ROSS, W. DNA fragments carrying genes for t RNA^{tyr}. Nature, 249: 738 (1974).

112. LINDAHL, T. Mammalian deoxyribonucleases acting on damaged DNA. In: "Molecular and cellular repair processes." Ed. Beers, R.F. Jr., Herriot, R.M., and Tilghman, R.C. The John Hopkins University Press. Baltimore, page 3 (1972).

ACKNOWLEDGMENTS

The author wishes to express her deep gratitude to Dr. L.S. Bates, her major professor, for his invaluable suggestions and encouragement throughout the course of this research and preparation of this thesis.

Appreciation and thanks are also extended to the members of her supervisory committee for their assistance in reviewing this work.

The author also wishes to extend special thanks to Freddy Parra for his assistance with the drawings, and to all the others who contributed in some measure to the successful culmination of this study.

Appreciation is also extended to CIEPE for financial support.

THE EFFECT OF ALIEN GERMPLASM ON 2M UREA SOLUBLE PROTEIN ELECTROPHORESIS

by

ELYZABETH CHAVEZ

B.S. Universidad Central de Venezuela, 1973

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Food Science
Department of Grain Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1977

ABSTRACT

Until just recently, morphological and cytological tests were the only criteria used for evolutionary, taxonomic and breeding studies. Sometimes it is difficult to differentiate between varieties by using only those characteristics. Protein electrophoresis constitutes a powerful approach to the problems of species relationships and permits one to make comparisons of numerous proteins. Due to the importance of the prolamines in cereals and to the fact they are synthesized under strict genetic control and independent of environmental effects, a method to differentiate between species and varieties by using prolamine starch gel electrophoresis was developed.

Electrophoretic patterns were established for the prolamines from different varieties of wheat, barley and rye and from the progenies of crosses between wheat x barley and barley x rye. Those progeny patterns reveal that some new proteins, not present in the parents are formed. This could be explained on the basis of interactions between genes, causing the synthesis of new prolamines in the hybrids.