

NATURE OF THE ENDOSPERM PROTEINS OF THE EXTRACTED TETRAPLOIDS
DERIVED FROM THREE COMMON WHEAT VARIETIES

A Thesis

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by

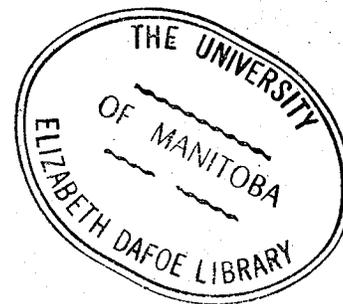
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Professor W. Bushuk

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ABSTRACT

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Major Professor: Dr. W. Bushuk

The protein compositions of AABB tetraploid wheat derived from three common wheat varieties, Prelude, Rescue and Thatcher, were compared with those of the hexaploid counterparts. The proteins were fractionated by solubility in appropriate solvents into albumins, globulins, gliadins, glutenins and insoluble residue proteins. The solubility characteristics showed that the tetraploid wheats of Rescue and Thatcher contained considerably more gliadin, more glutenin and less insoluble protein residue than the corresponding hexaploids. In contrast, Tetraprelude showed similar amounts of gliadin, glutenin and insoluble protein residue compared to its hexaploid.

Disc and starch-gel electrophoresis were used to separate and correlate the proteins in the four soluble fractions from related wheats. The electrophoretic patterns showed significant qualitative differences among the varieties studied, although the patterns of each extracted tetraploid and its hexaploid counterpart were quite similar.

Amino acid compositions of the flours for each extracted tetraploid and its hexaploid parent showed that the compositions are essentially the same. The amino acid composition varied significantly among the solubility fractions; however, the differences for any

one fraction among varieties were insignificant.

In addition to the extracted tetraploids and their parents, the proteins of five accessions of Ae. squarrosa, a synthetic hexaploid and its durum parent were studied. Differences in the electrophoretic patterns were found in the slow-moving gliadin proteins among the five accessions of Ae. squarrosa. Similar patterns were obtained for the synthetic hexaploid wheat and its durum wheat parent.

I INTRODUCTION

Common hexaploid wheat with the genomic formula AABBDD is an allopolyploid wheat. It has been established that the original ancestors of the three genomes A, B and D are the three diploid species T. monococcum, Ae. speltoides and Ae. squarrosa respectively (1). It is presumed that common hexaploid wheat originated through two widely separated evolutionary events. First, the AABB amphiploid resulted from a natural hybridization of the diploid species T. monococcum, and Ae. speltoides. Much later, the D genome of Ae. squarrosa was added, also by natural hybridization. Since both the durum ($2n=4x=28=AABB$) and the diploid wheats ($2n=2x=14=DD$) lack the breadmaking quality of the hexaploid bread wheats, the final addition of the D genome chromosomes appears to have contributed the genes for this quality.

Most of the wheat produced in the world is of the common hexaploid type, T. aestivum L. em Thell. ($2n=6x=42=AABBDD$). It is used primarily in the manufacture of flour for leavened baked products. For many centuries this crop has served as the basic food of man. Since wheat has been of such great economic importance to man himself, a great deal of effort has been devoted to research designed to answer the basic question: what properties of flour constituents determine its breadmaking quality?

It is now well established that the most important single constituent of bread wheat flour associated with its baking quality is its protein. Moreover, it has been recognized for some time that the breadmaking quality is determined by the very complex nature of the proteins which interact to form gluten.

In addition to the peculiar quality factors of the bread wheat proteins which give rise to baking quality, a relatively high quantity of protein is required to produce good bread (2).

In recent years, with the advent of various electrophoretic techniques, numerous workers have tried to associate differences in electrophoretic patterns obtained for various wheat varieties to differences in their breadmaking potentialities (3-5). Although marked differences in the patterns were observed, no direct relationship of particular protein bands to quality has been found. Thus, the genetic control of these proteins, and their relationship to the complex characteristic quality are far from being understood.

The extraction of the AABB components from three common wheat varieties Prelude, Rescue and Thatcher was recently accomplished in the Department of Plant Science, University of Manitoba. Breadmaking quality studies on the extracted tetraploid lines have demonstrated the importance of the D genome in the inheritance of breadmaking quality (6). The breadmaking quality of two of the extracted tetraploids, Rescue and Thatcher, was relatively poor, and not too unlike that of normal durum wheats. The tetraploid of Prelude showed better breadmaking quality than the other tetraploid lines, and was comparable to the three hexaploid wheats used as parents.

The genetic materials used in the present study were the three AABB tetraploid wheats derived from common bread wheats (hexaploids), Prelude, Rescue and Thatcher. They will be referred

to as Tetraprelude, Tetrarescue and Tetrathatcher respectively. The purpose of the study was to compare the proteins of the three extracted tetraploids and their hexaploid parents: 1) to determine if the removal of the D genome produced a deletion of certain proteins or a shift in the quantitative distribution of the proteins components which could be attributed to the loss of breadmaking quality, and 2) to determine if the proteins of Tetraprelude, "the durum type wheat with the high breadmaking quality," were similar to those of its hexaploid parent. Stewart 63 was included in the study as the representative of the natural tetraploid wheats with AABB genomic constitution.

In addition to the detailed investigation of the proteins of the extracted tetraploids and their parents, the proteins of two aneuploid lines involving chromosomes of the D genome of the common wheat variety, Chinese Spring, and five accessions of the species Ae. squarrosa and a synthetic AABBDD hexaploid were examined by gel electrophoresis. It was presumed that a study of these would provide additional information on the proteins whose synthesis is controlled by the D genome.

II LITERATURE REVIEW

1. Introduction

Wheat proteins have been of great interest to cereal chemists for over sixty years. This constituent of bread wheat flour determines to a large extent the physical properties of the dough, and in particular its breadmaking potential. It is generally accepted today that breadmaking quality is related primarily to the water-insoluble proteins of the flour which are collectively called gluten. In hydrated state, gluten, with its unique viscoelastic properties, forms the supporting three-dimensional network of the dough that is produced in the first step of the breadmaking process.

Berzelius in 1728 (7) was the first to describe the separation of gluten from wheat flour, but it was not until 1907 that Osborne (8) fractionated and classified these proteins on the basis of their solubility. He classified the proteins into four broad groups: 1) water-soluble proteins or albumins; 2) salt-soluble proteins or globulins; 3) alcohol-soluble proteins or gliadins; and 4) proteins soluble in dilute acid or base, the glutenins. Moreover, Osborne reported that gluten comprised about 80% of the proteins in flour. Gluten comprised two main groups of proteins, gliadins and glutenins, present in nearly equal amounts. With the advent of modern techniques of protein chemistry such as the analytical ultracentrifuge and electrophoresis, it was soon shown that each of the four protein fractions suggested by Osborne was a heterogenous mixture of a

large number of molecularly different proteins.

2. Electrophoresis Studies of Wheat Proteins

Electrophoretic separation of proteins was first reported by Tiselius in 1937 (9). However, this technique was not applied to the separation of the wheat proteins until 1944 (10). In recent years, both moving-boundary and zone electrophoresis have been successfully applied to studies of cereal grain proteins. Zone electrophoresis requires the use of various physical support materials such as filter paper or gels on which the protein migrates while in moving boundary electrophoresis, the proteins move freely in a buffer solution.

Filter paper and agar gels were the first support materials used for separation of proteins by zone electrophoresis (11-12). However, these materials have found very limited application in the separation of cereal proteins. A major development in zone electrophoresis occurred in 1955 when Smithies introduced the use of starch gels as support material for the separation of serum proteins (13). The resolution obtained by starch-gel electrophoresis was much superior to that obtained by other types of zone electrophoresis. Elton and Ewart (14) were the first to use this support material, in conjunction with the aluminum lactate buffer system of Jones et al (15), for the separation of flour proteins. In 1961 Woychik et al (16) improved the separation of flour proteins by starch-gel electrophoresis by the addition of urea to the gel

to increase the solubility of the proteins.

Polyacrylamide gels were introduced for zone electrophoresis by Raymond and Wang in 1960 (17) and adapted to wheat proteins by Lee in 1962 (18). In contrast to starch gels, polyacrylamide gels are transparent, thermostable, non ionic, and their pore size can be readily varied over a wide range by simply changing the concentration of acrylamide used to prepare the gels (19). The electrofocusing technique for separating wheat proteins recently developed by Wrigley (20) also employs polyacrylamide gels as the supporting material.

3. Water and Salt-Soluble Proteins

The water-soluble proteins or albumins and the salt-soluble proteins or globulins of wheat are collectively referred to as the soluble proteins. The first comprehensive study of these proteins was that of Osborne (8). Subsequent investigations prior to 1944 have been reviewed by Bailey (21). In recent years, Pence and coworkers (22-24) have extensively studied these proteins. Most of the early findings were subsequently modified and refined by new techniques of protein chemistry.

Finney (25) reported that the water-solubles were necessary for normal baking performance in two of his three reconstituted flours. In 1951, Pence et al (22) found that water-soluble components from flours with widely different baking characteristics were required for maximum performance of flours reconstituted from all glutens, except the one from durum wheat. A crude albumin

component isolated from the water-solubles produced a positive volume response, and reduced the mixing time. In 1962 Pence (26) concluded that albumin proteins are implicated in the baking performance of the flour. Furthermore, he suggested that differences in soluble constituents might account for the variations in baking quality of different flours.

Recently, Hosney et al (27) reported that the water-soluble fraction of the flour was not involved in breadmaking quality differences among varieties, although it was required to produce a normal loaf of bread. It was found that the water-solubles contributed to gas production and affected the physical properties of the dough. However, the albumins and globulins were not involved in the breadmaking quality since removal of these proteins from the water-soluble fraction did not decrease the loaf volume of the bread baked from reconstituted flours. On the basis of their reconstitution studies, Hosney et al (27) suggested that soluble pentosans and glycolipids were the water-soluble constituents that were essential for baking performance.

There have been numerous studies of the physicochemical properties of the soluble proteins of wheat. Laws and France (28) reported in 1948 that a water extract from three flours of different breadmaking quality gave similar patterns in moving boundary electrophoresis. In 1953, Pence and Elder (23) separated the albumins by paper electrophoresis into at least six individual components of similar molecular size. The albumins were characterized by a

higher tryptophan content, but a lower amide nitrogen content than the other wheat proteins. The albumins had an apparent molecular weight of about 28,000 in dilute salt solution, but in the presence of urea or sodium salicylate the molecular weight decreased to about 20,000. A subsequent study by Pence (29) indicated that as many as eleven water-soluble protein components could be detected by paper electrophoresis. The approximate isoelectric points of the eleven individual components varied from 4.5 to 8.7. In 1954 Pence et al (30) demonstrated distinct differences between the compositions of the albumins of durum and common wheat flours. These differences in component distribution appeared to be unaffected by differences in growth location and crop year.

In 1954 Pence et al (24) determined the soluble protein content of 32 flours of widely different type and baking quality. The total soluble protein contents varied in the range, from 13 to 22% of the total flour protein. The amounts of albumins were generally slightly higher than the globulins. Both the albumin and globulin contents, as well as the ratio of the two, varied significantly among the flours.

More recently, Holme (31) separated the albumin fraction from cake flour into at least five components by moving boundary electrophoresis. Elton and Ewart (32) examined the albumins and globulins from 8 wheat varieties by starch-gel electrophoresis. No significant differences were observed in their electrophoretic patterns. Woychik et al (16) separated the water-soluble proteins into at

least 9 components on starch gel in the presence of urea. Later, Elton and Ewart (33) improved the resolution of their starch-gel electrophoresis technique and reported some minor differences in the electrophoretic patterns of the water-soluble proteins from several wheat varieties. In 1963 Nimmo et al (34), using polyacrylamide-gel electrophoresis, found at least 15 protein components in a water extract of bread wheat flour.

Silano et al (35) used disc electrophoresis to analyze the albumin and globulin fractions of several varieties of T. aestivum and T. durum. Fourteen albumin and 15 globulin bands were observed. Varietal differences were found, particularly in the fast moving albumin bands.

Recently, Feillet and Nimmo (36) isolated and characterized two albumins (13A and 13B) from bread flour. Albumin 13B had a relatively high valine content, and no histidine or phenylalanine while albumin 13A contained all the common amino acids and had a relatively high content of alanine. No free sulfhydryl groups were found in either protein. Molecular weights of albumin 13A and albumin 13B were 24,800 and 13,950, respectively, determined from sedimentation-equilibrium data.

Relatively little is known about the salt-soluble proteins (globulins) of wheat flour. So far, no functional role has been attributed to these proteins, although Pence (26) suggested that they may be involved in breadmaking performance. Pence (26) was unsuccessful in his attempts to reconstitute flour from which globulins had been removed. It was not possible to ascertain

whether it was the deletion of the globulins that produced the observed detrimental effect or whether the salt used in the extraction of the globulins damaged the protein system of the flour.

In 1949 Danielson (37) observed two distinct globulins, gamma and alpha, in salt solution extracts of flour by means of the analytical ultracentrifuge. According to this investigator, gamma globulin has a molecular weight of about 210,000. Three different globulin components were found by Pence and Elder (23) in preparations from flour and defatted wheat germ. These globulins were characterized by relatively low tryptophan and amide nitrogen contents, and a relatively high arginine content when compared with the total flour protein. Total globulin content of wheats of different types ranged from 5 to 11% of the total flour protein. Elton and Ewart (32) detected two globulin bands which migrated most rapidly in starch-gel electrophoresis of dialyzed salt extracts of flour. Recently, Fisher et al (38) identified the fast-moving globulin doublet found by Elton and Ewart (32) as purothionin.

4. Gluten or Insoluble Proteins

Finney demonstrated in 1944 (25) that wheat gluten is the fraction of flour that is primarily responsible for its breadmaking quality. Since that time, many attempts have been made to detect differences in the gluten fraction that could be related to differences in baking quality of the flour from which the gluten was obtained. The separation and characterization of the gluten

proteins has been hampered by the lack of suitable solvents capable of solubilizing all the proteins comprising gluten. A major advance was made in 1959 when Jones et al (15) discovered that aluminum lactate buffer of pH3.1 could be used in electrophoresis to separate gluten proteins.

Gluten is normally prepared by washing dough in a stream of water or a dilute salt solution until the starch and solubles are removed. Crude gluten contains about 85% protein, 8.3% lipid, 6% starch, and 0.7% ash, (39). The protein component of gluten is mainly gliadin and glutenin present in approximately equal amounts.

The unique elastic and cohesive properties of doughs are usually associated with analogous properties of the glutenin fraction (15). The glutenin group appears to be a mixture of a number of molecular species which contain a wide range of molecular sizes with the molecular weight ranging from 50,000 to over 2 million (40). Reduction of the glutenin disulfide groups decreases its molecular weight to approximately 20,000 (41).

In starch-gel electrophoresis, glutenin does not migrate into the gel due to its large size (16), although, upon reduction it can be separated into approximately 20 components (42). Seven of the components of reduced glutenin migrated at the same rate as components of reduced gliadin suggesting that the gliadins might be linked by S-S bonds to form glutenin. Elton and Ewart (43) reduced the glutenin from four wheats and separated the components on starch gel. The electrophoretic patterns showed that there were

varietal differences among the reduced glutenins. Huebner et al (44) compared the glutenins isolated from five different classes of wheat. Electrophoretic patterns of the reduced-alkylated glutenins showed significant differences among varieties of the same class, but the greatest differences were among different classes of wheat. Reduced-alkylated glutenin from durum wheat contained few or none of the slow moving components present in the bread wheat glutenins that were examined.

Gliadin, the alcohol-soluble proteins of wheat forms an integral part of gluten and plays an important role in determining the breadmaking potentialities of flour (45). It is highly heterogeneous; the actual number of revealed components depends on the analytical technique used for their separation. Its average molecular weight by sedimentation-diffusion was found to be about 45,000 (46) and its isoelectric point at a pH of about 6.5. Gliadin group of proteins is characterized by extremely high glutamine and proline contents (47). It has a much lower intrinsic viscosity than the glutenins probably because of its smaller size and a more compact globular conformation (48). There is considerable evidence from studies of reduced gliadin that its disulfide bonds are intramolecular (49).

Schwert et al (10) found no differences in the electrophoretic patterns of gliadins from a variety of flour prepared by a number of different methods. Laws and France (28) reported that no significant differences could be detected by moving boundary electro-

phoresis of wheat gluten proteins including gliadins derived from different wheats. In 1954 Mills (50) reported that at least four protein components were present in the gliadin group.

Jones et al (15) used aluminum lactate buffer solution in conjunction with moving boundary electrophoresis to separate gluten proteins into four major and one minor components. Further examination of the electropherograms from four bread wheats (two with good and two with poor baking qualities) showed the same protein composition. However, glutens from two durum wheats, examined similarly, showed quite different electropherograms.

In 1961 Cluskey et al (51) separated by moving boundary electrophoresis protein components of gluten and water solubles from hard and soft wheat flours. On the basis of electrophoretic mobility they designated the gluten proteins into five components identified by Greek letters alpha, beta, gamma, omega, and delta. Hard wheat flours contained more alpha, gamma, and omega components but the same amounts of beta and gamma, the fast moving components. They were able to distinguish between hard and soft bread wheats on the basis of protein composition, although the differences were small.

Woychik et al (16) resolved gluten proteins into nine distinct components by starch-gel electrophoresis in the presence of urea. Eight of the nine components were attributed to the gliadin group. In 1962 Elton and Ewart (32) reported significant differences in

the starch-gel electrophoresis patterns for gluten proteins of different bread wheats. They attributed these to real differences in the proteins. It was suggested that the observed differences might be sufficient to account for the differences in breadmaking quality of the wheats examined. Later the same authors (33) showed that both qualitative and quantitative differences existed between starch-gel the patterns or the gliadins from several wheat varieties.

Lee and Wrigley (5) compared the gluten proteins of eight commercial wheat varieties and four tetraploid wheats by ion-exchange column chromatography and by polyacrylamide-gel electrophoresis. Definite differences in protein profiles for the commercial wheats and tetraploid wheats were observed. However, it was not possible to relate a type of chromatographic pattern to baking quality of the eight commercial wheat studied.

In 1963, Graham (52) using an improved apparatus and procedure for starch-gel electrophoresis, showed that similar protein components were extracted with widely different solvents. Similar patterns were obtained for proteins from similar genotypes while different genotypes showed marked differences in the slow moving or gliadin components. Using the same technique, Coulson and Sim (3) examined the proteins from 34 different varieties of T. aestivum. Major differences in protein patterns were found in the gliadin proteins while the patterns for the fast moving albumin and globulin components were similar for all varieties examined.

Furthermore, there was no obvious correlation between the proteins patterns and the physical or baking-quality characteristics of the flour.

Lee and Ronald (53) studied the effect of environment on wheat gliadin in four T. aestivum varieties grown in Australia under a wide range of growing conditions. Varietal differences in the distribution of gliadin components were found to be much more significant than the differences that could be attributed to environmental factors.

More recently Huebner et al (54) compared the compositions of the gliadin group of proteins from a number of varieties of common, durum, and club wheats by column chromatography and by starch-gel electrophoresis. Greatest differences were observed for varieties representing different classes while differences among varieties of the same class were small.

Using starch-gel electrophoresis, Doekes (4) classified 80 varieties and selected lines of wheat into 5 main groups having similar electropherograms. Group I comprised soft winter wheats with poor baking quality while group V included hard red wheat varieties of relatively good breadmaking quality. Furthermore, group I varieties showed a compact gliadin pattern while group V displayed a gliadin pattern in which the components showed a broad range of mobilities. Groups II, III and IV included

varieties with diverse origins, and considerable variation in baking quality. Their gliadin patterns were intermediate between those of group I and V.

5. Amino Acid Composition

Recent development of highly efficient and rapid analytical techniques for determination of amino acid composition of proteins has produced accurate amino acid analyses of wheat proteins. Although, these data have been extremely useful in relation to nutritional quality of wheat proteins, they are of limited value in relation to the breadmaking quality of wheat varieties (2). Amino acid compositions for a wide variety of wheats that differ widely in baking quality were found to be essentially the same (55).

Glutamine is the predominant amino acid present in wheat proteins, and proline is also present in relatively high amounts (56). Together they make up approximately 50% of the amino acids in gluten proteins (55). Isoleucine, leucine, phenylalanine, serine, tyrosine and valine contents are relatively high in gluten compared to other proteins (55). The soluble proteins generally contain more cystine, tryptophan, basic amino acids and glutamic acid than the gluten proteins (57). The albumins contain more cystine and cysteine than the globulins or gluten proteins.

Glutamic acid and proline contents have been shown to increase, while lysine tends to decrease as the protein content of wheat increases (55). Lawrence et al (58) found that lysine increased

significantly as the protein content decreased below 13.5%. However, for wheats with more than 13.5% protein, there was no significant correlation between lysine and protein contents.

In 1961 Woychik et al (59) determined the amino acid compositions of six protein components isolated from wheat gluten. While their compositions were similar, significant differences were observed which indicate the individuality of these proteins.

Ewart (60) compared the amino acid compositions of the glutenins and gliadins from two strong and two weak wheats. The compositions of the glutenins were similar to those of the gliadins. There were minor differences in the amounts of some amino acids. No significant differences in composition were observed for varieties of the same class or for strong and weak wheats.

Recently, Tkachuk (56) determined amino acid composition of six flours which differed widely in their breadmaking quality. The compositions for the six flours were essentially the same.

6. Genetic Inheritance of Protein Components in Bread Wheat and Related Species

In recent years, a large number of reports have appeared in the literature of attempts to relate electrophoretic or chromatographic patterns for the proteins to genetic relationships among wheats and related grain species. For example, Johnson and Hall (61) used polyacrylamide-gel electrophoresis to obtain evidence of the genetic

origin of bread wheat. The electrophoretic patterns for extracts from T. monococcum, T. dicoccum, and T. aestivum showed that the A and B genomes contributed different proteins. Some evolutionary divergence had apparently occurred in the A genome of T. dicoccum since the proteins of this species were only partially homologous with those of T. monococcum.

Hall et al (62) examined the genome relationships in the Triticinae in terms of protein homology based on electrophoretic patterns. Different degrees of homology were found for the A, B, and D genomes. In 1967, Johnson et al (63) compared the disc electrophoretic patterns for protein extracts from 26 hexaploid and 64 tetraploid wheats. Homology was observed for nine albumin bands in the two groups of wheat examined. Three additional albumin bands in the hexaploids were attributed to the D genome. In addition, differences in the patterns for the slow moving protein bands of the gliadin group were found among (the tetraploid species, and also among the hexaploid subspecies studied) varieties within each group.

The identification of wheat chromosomes involved in the control of breadmaking quality has been hampered by the polyploid nature of wheat. Accordingly, aneuploid and substitution lines have been used to determine the chromosomes which control flour properties that are important in baking quality. A number of chromosomes, mostly of the B and D genomes, have been reported to be involved in the genetic control of breadmaking quality (64-66).

Welsh and Hehn (64) examined the quality of the complete set of monosomic lines of the hard red winter wheat, Kharkof MC22. They observed that chromosome 1D resulted in a drastic reduction of the doughball fermentation time (Pelshenke Test) and an extreme weakening of the farinograph curve.

Morris et al (65) and Schmidt et al (66) studied chromosome substitution lines of the hard red winter variety Cheyenne into the variety Chinese Spring. Mixograph and bake tests indicated that chromosomes 1B, 4B, 7B and 5D were primarily responsible for the high baking quality of Cheyenne. Recently, Welsh et al (67) examined the milling and baking qualities of the complete sets of substitution lines of three hard red spring wheat varieties, Thatcher, Hope and Timstein with Chinese Spring as the common background variety. Only chromosome 2A of Thatcher produced a marked change in the baking properties of the flour from the background variety. On the other hand, 11 Hope and 16 Timstein chromosomes produced notable change. Chromosome 2B of Hope and chromosomes 3B and 6B of Timstein had the greatest effects. On the basis of current state of knowledge, it appears that the genes for breadmaking quality are located on a number of different chromosomes.

There have been a number of reports on the effects of additions or removals of genomes, and of specific chromosomes on the proteins of bread wheats and related species. In 1967, Boyd and Lee (68) reported similar patterns were obtained by starch-gel electrophoresis for protein extracts from single seeds of the

hexaploid wheat Canthatch and its extracted AABB tetraploid, Tetra-canthatch. In a subsequent paper, Boyd and Wrigley (69) reported that the AABB Tetra-canthatch lacked at least 4 protein bands present in its hexaploid parent. It was indicated that in the earlier study, two samples of the hexaploid parent variety instead of the tetraploid and the hexaploid were examined due to a labelling error. It appears, therefore, that removal of the D genome from the hard red spring wheat variety Canthatch decreases the number of protein components.

In a study of the appropriate ditelocentric aneuploid lines of Chinese Spring, Boyd and Lee (68) showed that removal of the 1D chromosome resulted in a loss of two slow moving protein bands in starch-gel electrophoresis patterns. With the same technique Shepherd (70) examined the endosperm proteins of compensating nullisomic-tetrasomic lines of Chinese Spring and found that nulli 1D-tetra 1A and nulli 1D-tetra 1B lacked two similar slow moving bands. These results agreed with the previous suggestion of Boyd and Lee (68) that the genes for the two missing proteins are on chromosome 1D. Of the 33 nullisomic-tetrasomic lines of Chinese Spring studied by Shepherd (70), 9 of the 17 major protein band of Chinese Spring were accounted for by the removal of individual chromosomes. Presumably the synthesis of the remaining eight components is controlled by diploid or triploid factors on more than one chromosome pair.

Further evidence of the importance of the D genome, and specifically the 1D chromosome, of hexaploid bread wheats to bread-

making quality was reported by Kaltsikes et al (6). For this study, the AABB tetraploid components were extracted from three hard red spring wheat varieties, Prelude, Rescue, and Thatcher. The bread-making qualities of the extracted tetraploid of Rescue and Thatcher were relatively poor, and not too unlike that of normal durum wheats. On the other hand, tetraploid Prelude showed much superior bread-making quality than the other two extracted tetraploids, and was comparable in quality to the three hexaploid parent varieties. This higher quality of Tetraprelude was attributed to two factors: 1) a somewhat higher protein content; and 2) a translocation involving the long arm of chromosome 1D, to a chromosome of either the A or the B genome.

Recent studies of Kerber and Tipples (71) provide further evidence of the importance of the D genome in controlling the baking quality of bread wheats. Results of this study were similar to those of Kaltsikes et al (6). Extracted AABB tetraploid of Canthatch had extremely poor baking quality compared with the hexaploid Canthatch. Five synthetic hexaploids produced by combining the extracted AABB tetraploid of Canthatch with different varieties of Ae. squarrosa which supplied the D genome were superior in baking qualities than the extracted tetraploid but still considerably inferior to Canthatch.

7. Proteins of Alien Genome Combinations

Yong and Unrau (72) used starch-gel electrophoresis to compare the proteins of hexaploid AABRRR Triticale (durum wheat x rye) and

its parental species. These authors reported that the synthetic hybrid had four "new" proteins not present in the parents. It was postulated that the "new" proteins resulted from an interaction between the alien parental genomes. Barber et al (73) compared the esterases of one line of Triticale and its two parents, and found one "new" esterase band in the synthetic amphiploid.

The proteins of the same strain of Triticale and its parents as used by Yong and Unrau (72) were recently re-examined by Chen and Bushuk (74) using a more elaborate separation and electrophoretic technique. No "new" proteins were detected. Chen and Bushuk (74) concluded that the proteins in the Triticale studied were directly inherited from its parental species. Similar results were obtained by Johnson and Hall (61) who examined the proteins of an octaploid AABBDDRR Triticale and its two parental species, T. aestivum (AABBDD) and S. cereale (RR). The polyacrylamide-gel electrophoresis pattern for the Triticale was essentially the same as the pattern for a mixture of extracts from the parent species durum wheat and rye. The discrepancy between the results of Yong and Unrau (72) and Barber et al (73) on the other hand, and those of Johnson and Hall (61) and Chen and Bushuk (74) on the other remains to be clarified.

III MATERIALS

Three common bread wheat varieties Prelude, Rescue and Thatcher, their extracted AABB tetraploids Tetraprelude, Tetrarescue, and Tetrathatcher, and a durum variety, Stewart 63, were used in this investigation. The tetraploids were produced by Kaltsikes (75) using the back-crossing technique developed by Kerber (76). Genetic extraction of the tetraploids from hexaploid wheats was accomplished as follows. The variety Prelude will be used to illustrate the technique. First a cross was made between the common wheat variety Prelude and the durum wheat variety Stewart 63. The resulting pentaploid was backcrossed several times to Prelude, which after selfing gave a tetraploid wheat with the AA and BB genomes derived from Prelude. The seven wheats used in this part of the study were grown together in 1967.

The materials used in the second part of this study, were the disomic parent and the aneuploid lines nulli 1D-tetra IA and nulli 1D-tetra 1B, of the common wheat variety Chinese Spring, a synthetic AABBDD hexaploid wheat [T. durum (Iumillo x Ae. squarrosa)] and five species of Ae. squarrosa, one of the diploid progenitors of common wheat. The seeds of Chinese Spring and its aneuploids were provided by Dr. Feldman of the Plant Science Department, University of Manitoba, while the Ae. squarrosa seeds and the synthetic hexaploid were part of the University of Manitoba collection. Since there was not sufficient material of this group of samples to mill into flour, their proteins were extracted from single seeds.

IV METHODS

1. Preparation of the Flour

The wheat (200 g.) was tempered to 15.5% moisture and milled into flour on a Buhler experimental mill. The flour was stored at 4°C in sealed containers.

2. Extraction of the Proteins from Flour

Flour proteins of the extracted tetraploids and their hexaploid counterparts were fractionated by the classical technique of Osborne (8). By this procedure five broad fractions are obtained: 1) water-soluble proteins or albumins; 2) salt-soluble proteins or globulins; 3) alcohol-soluble proteins or gliadins; 4) acetic acid-soluble proteins or glutenins; and 5) the insoluble-residue proteins. The fractionation procedure is summarized in Fig. 1.

All extractions were in duplicate and were made in a cold room at 4°C to minimize enzymic activity and the possibility of denaturation. The flour-solvent slurry was continuously stirred in 250 ml. centrifuge bottles with a magnetic stirrer throughout the extraction period. At the end of each extraction period, the mixture was centrifuged at 3,000 rpm. (1860xg.) for 30 minutes. The supernatant was removed and the residue was extracted as above with the subsequent extraction solvent.

3. Water-and Salt-Soluble Proteins

Initially, 10g. (dry weight basis) of flour was extracted with 70 ml. of 0.5M sodium chloride (salt) solution for 2 hours,

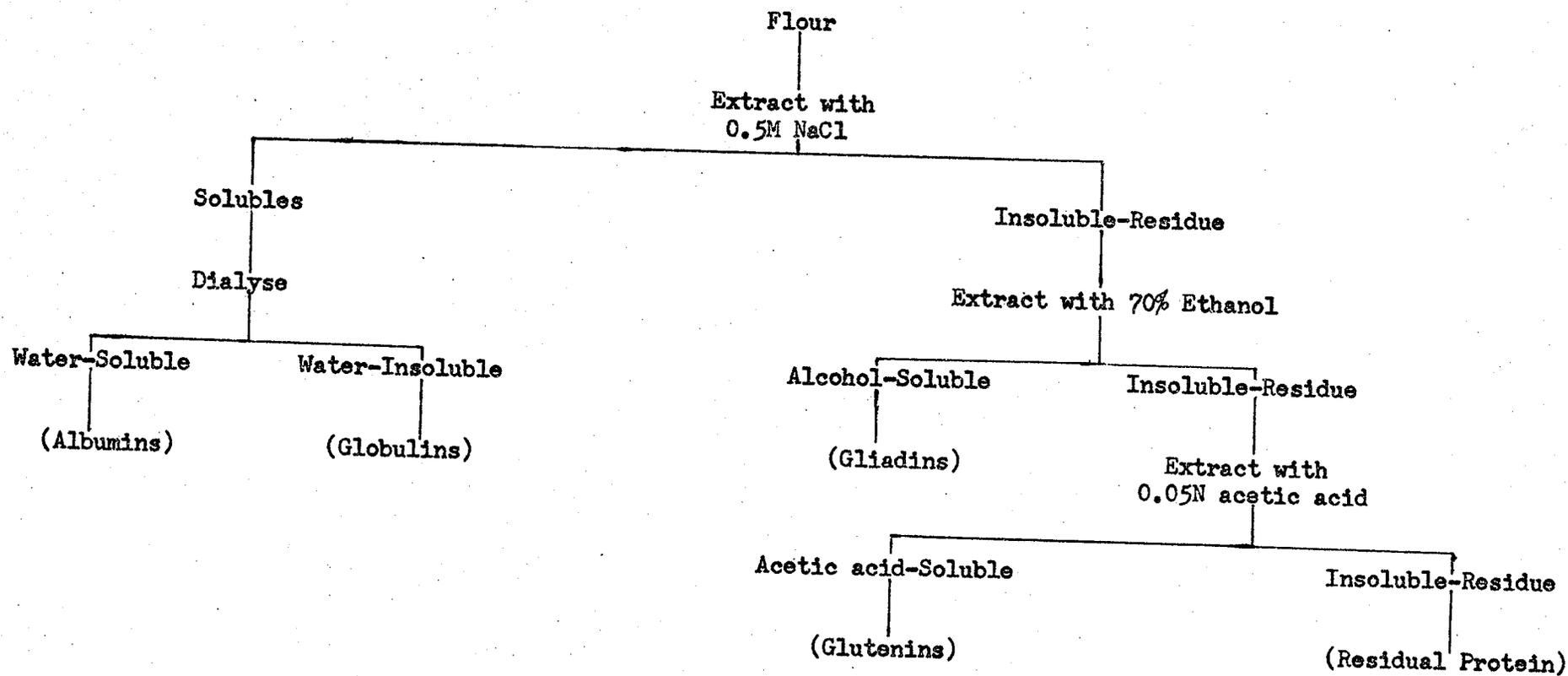


Fig. 1. Solubility fractionation of the flour proteins

followed by a second extraction with 40 ml. of 0.5M salt solution for 1 hour, and a third extraction with 40 ml. of water for 1 hour to remove the residual salt. The three supernatants were combined and dialysed against 4 liters of distilled water for 48 hours. Four changes of fresh distilled water were used in the dialysis. After dialysis, the suspension was centrifuged for 30 minutes to separate the precipitated salt-soluble proteins. The precipitate was washed several times with water, and recentrifuged. The supernatants obtained from all the centrifugations were combined. This contained the water-solubles which will be designated as the water-soluble fraction. The remaining precipitate will be referred to as the salt-soluble fraction.

4. Extraction of Alcohol-and Acetic Acid-Soluble Proteins

The residue obtained from the salt solution and water extractions described above was extracted for 2 hours with 70% ethanol solution, followed by a second 1 hour extraction with 40 ml. of the same ethanol solution. The supernatants obtained were transferred into a 250 ml. round bottom flask. The alcohol was removed from the supernatants under reduced pressure on a rotary evaporator. This fraction will be designated as the alcohol-soluble fraction.

The residue remaining after the ethanol extraction was extracted for 2 hours with 70 ml. of 0.05N acetic acid solution followed by a second extraction for 1 hour with 40 ml. of 0.05N acetic acid solution. The two supernatants were combined to give the acetic acid-soluble fraction. The remaining material will be

referred to as the insoluble-residue fraction.

All five fractions obtained were transferred into weighed trays and frozen. The samples were lyophilized, and the weights of the dried materials were determined for calculation of the yield of various fractions. The dried fractions were transferred into plastic air-tight bottles and stored in a desiccator at -20°C .

5. Determination of Protein Content

Protein contents ($N \times 5.7$) of the flour and the various solubility fractions were determined using the standard macro Kjeldahl and micro Kjeldahl procedures respectively.

6. Starch-gel Electrophoresis

Starch-gel electrophoresis was carried out in the presence of 2M urea in 0.1μ aluminum lactate buffer of pH 3.2. Aluminum lactate was prepared according to Jones and Cluskey (77). To prepare the gel, 65 g. of hydrolyzed starch (Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada) was suspended in 550 ml. of the buffer-urea solution. One ml. of 1% ethylmercurithiosalicylic acid, sodium salt (Thimerosal) was added to the gel mixture to inactivate beta amylase activity during electrophoresis (78). The mixture was heated in a water bath with mild continuous stirring until the temperature reached 75°C . Vigorous stirring was avoided since this produced large air bubbles in the slurry which, on cooling, gave a non-uniform gel. When the temperature reached 75°C . the liquid was poured into a 12 x 30 x 0.6 cm. plexiglass gel tray. The sample slots in the gel were cast using a six-position slot former placed at one end of the tray. The

starch slurry was allowed to cool for 2 hours and then stored in a cold room at 4°C. for 22 hours. The following day, the slot former was carefully lifted from the gel. Finally, the excess gel was removed from the top of the plate by slicing with a cutter made from a thin stainless steel wire.

For the electrophoresis experiment, 0.2 ml. of a 1% protein solution or extract from a single seed was applied to each slot and the slot covered with a glass cover slip. During the experiment, the entire gel was covered with saran wrap to prevent excessive drying out.

The horizontal starch-gel arrangement was used in the entire investigation. The gel was connected to the buffer solutions in the electrode boxes by several sheets of Whatman No. 1 filter paper. Electrophoresis was conducted in a cold room at 4°C. to prevent build up of excessive heat. Each run was for six hours at a potential of 6 volts per cm. At the conclusion of the electrophoresis, the gel was carefully removed from the tray and the top 2mm. of the gel was sliced off with the wire cutter and discarded. The lower part of the gel was stained overnight in 200 ml. of 2% trichloroacetic solution containing 10 ml. of 0.7% nigrosin solution. The gel was removed from the staining solution, washed with water, and photographed.

7. Disc Electrophoresis

Disc electrophoresis on polyacrylamide gel was carried out in manner similar to that of Davis (79). The apparatus used was

purchased from Buchler Instrument Inc. In this apparatus, 12 tubes can be run simultaneously. Stock solutions for preparing the gels were made up as shown in Table 1.

The lower gel was prepared by mixing 0.3 ml. of acrylamide solution, 0.6 ml. of buffer solution and 0.3 ml. of catalyst solution per tube. This gave concentration of 7.5% of acrylamide. The gel mixture was covered with a layer of water and photopolymerized for 30 minutes. Excess water was decanted.

The upper gel, comprising 0.1 ml. of buffer solution, 0.05 ml. of acrylamide solution and 0.05 ml. of catalyst solution per tube, was photopolymerized for 15 minutes. After polymerization, the tubes were placed into the upper chamber of the electrophoresis apparatus. The protein solution (25 to 50 μ l of a 1% (W/V) in 15% sucrose solution) was then added to each tube. The upper or the anode chamber contained 500 ml. of glycine-acetic acid buffer solution of pH 4.0 while the lower or the cathode chamber was filled with 500 ml. of acetic acid-potassium hydroxide buffer solution of pH 4.3. Electrophoresis was carried out in a water cooled apparatus with a current of 4 ma. per tube applied for approximately 2 hours. Methyl green was used as the visual marker. After completion of the electrophoresis, the gels were removed from the tubes and stained for several hours in 0.5% amido black in 7% acetic acid solution saturated with mercuric chloride. The unabsorbed dye was removed from the gel electrophoretically. The gels were stored in small test-tubes containing 7% acetic acids solution

Table 1. Solutions for Preparation of Gels for Disc Electrophoresis

	<u>Lower gel</u>	<u>Upper gel</u>
<u>Catalyst solution</u>		
Ammonium persulfate	60 mg.	60 mg.
Riboflavin	4 mg.	2 mg.
Water	to 100 ml.	to 100 ml.
<u>Acrylamide solution</u>		
N, N - methylene bisacrylamide	0.8 g.	0.8 g.
Acrylamide	30 g.	10 g.
Water	to 100 ml.	to 100 ml.
<u>Buffer solution</u>		
1N potassium hydroxide solution	24 ml.	48 ml.
Acetic acid (glacial)	53.2 ml.	3.65 ml.
Temed*	0.48 ml.	0.2 ml.
Water	to 100 ml.	to 100 ml.

*Temed = N, N, N', N' - tetramethylethylenediamine

during the time required for visual comparison and photographing.

8. Amino Acid Analyses

Amino acid compositions were determined for the flours and the four freeze-dried fractions for each variety or line of grain used in the first part of this study. The analyses were made on a Beckman-Spinco Model 120 Automatic amino acid analyzer using the method of Spackmann et al (80). The amount of each amino acid was determined by comparing the recovery of the particular amino acid with that of the same acid using a standard amino acid mixture.

For preparation of hydrolyzates, the quantity of material containing 20 mg. of protein was mixed with 3 ml. of twice distilled 6N hydrochloric acid in a 17 mm. test tube. The contents of the test tube were frozen and the tubes evacuated and sealed. Hydrolysis time was 18 hours at 110°C. After hydrolysis, the contents of the test tube were frozen and the tube was opened. The frozen hydrolyzates were placed in a desiccator containing solid sodium hydroxide and then evacuated to remove the HCl. Finally, the amino acids were dissolved in a known amount of citrate buffer and an aliquot was applied to the ion-exchange column of the amino acid analyzer.

9. Extraction of Proteins from Single Seeds

For the analysis of proteins in individual seeds used in the second part of this study, the protein solutions were prepared by

extracting ground endosperm with 2M urea or 4M dimethylformamide solution in 0.1N acetic acid solution for 24 hours at 4°C. in 15 ml. centrifuge tubes. The suspensions were centrifuged, and proper aliquots of the supernatant examined by starch-gel or disc electrophoresis.

10. Reduction of Disulfide Bonds of Flour Proteins

Reduction of the disulfide bonds was carried out at room temperature using 1% protein solution in 0.1M phosphate buffer of pH 8.0 containing 6M urea by treatment with 2-mercaptoethanol at a concentration approximately 100 times the disulfide content. After 30 minutes, the liberated sulfhydryl groups were alkylated for 15 minutes by adding a five-fold excess of acrylonitrile. The solution containing the reduced alkylated proteins was adjusted to pH 4.0 with acetic acid, dialysed against 0.1N acetic acid for 48 hours and then freeze-dried.

V RESULTS AND DISCUSSION

1. Breadmaking Quality

The extracted tetraploids and their hexaploid parents used in this study had been previously subjected to a large number of tests to evaluate their baking quality by Kaltsikes et al (6). Since the materials used in the present investigation were from the original seed stocks produced by Kaltsikes, it was felt that the original quality data might be of interest to cereal technologists and plant breeders. Accordingly, the data originally obtained by Kaltsikes et al (6) is included in this thesis as Appendix I.

The results of Kaltsikes et al (6) can be summarized as follows:

- (1) Each extracted tetraploid had a higher protein content and gave a lower flour yield than its hexaploid parent.
- (2) The dough mixing curves for Tetrarescue and Tetrathatcher were weaker than those of their corresponding hexaploids. Tetraprelude had a stronger mixing curve than the other tetraploids but it was somewhat weaker than that for the hexaploid Prelude.
- (3) Tetrarescue and Tetrathatcher had inferior breadmaking quality (considerably lower loaf volume) than their hexaploid counterparts. Tetraprelude, on the other hand, gave a higher loaf volume than the other tetraploids and was comparable in this characteristic to the hexaploid Prelude and the other hexaploid wheats.

Table 2. Protein and Moisture Contents, and Flour Yields for the Three Extracted AABB Tetraploid Wheats, Their AABBDD Hexaploid Parents and Stewart 63 (a Durum Wheat).

	<u>Protein*</u> %	<u>Moisture</u> %	<u>Extraction</u> %
Tetraprelude	15.9	12.6	63.0
Prelude	14.8	12.9	80.0
Tetrarescue	16.4	12.6	50.0
Rescue	13.1	13.1	77.5
Tetrathatcher	14.5	12.7	50.0
Thatcher	14.3	13.2	74.0
Stewart 63	13.3	13.4	70.0

*Protein contents are on a dry basis.

2. Milling of Flour

Protein and moisture contents of flour milled from 200 g. of wheat of the three extracted tetraploids, their parents and a durum wheat variety Stewart 63 are given in Table 2. These results are essentially the same as those published by Kaltsikes et al (6) for the same wheat samples. As noted previously (6), Tetraprelude and Tetrarescue had a higher protein content than their hexaploid parents while Thatcher and Tetrathatcher had similar protein contents. The hexaploid wheat gave a higher flour yield than their extracted tetraploids.

3. Solubility Fractionation

At the outset of the present study it was decided to separate and characterize the proteins of the three extracted tetraploids and their hexaploid parents according to the Osborne (8) classification of cereal proteins. Extraction conditions were carefully controlled throughout to ensure that quantitative and qualitative differences between fractions for a particular wheat sample and between wheats for a particular fraction would not be masked by variations in the fractionation procedure.

4. Solubility Distribution on a Weight Basis

The total weights of extractable material expressed in percent of the total material recovered, are given in Table 3. Recoveries of the total weight of the original flour were between 92.6 and 97.6%. The incomplete recovery could be attributed to losses during dialysis of low molecular weight compounds that are extracted in the salt fraction.

Table 3. Solubility Distributions of Flours from Extracted Tetraploid Wheats and Their Hexaploid Counterparts

	Tetra- thatcher	Thatcher	Tetra- prelude	Prelude	Tetra- rescue	Rescue	Stewart 63
	%	%	%	%	%	%	%
Water-soluble	4.5	3.2	3.4	3.2	5.0	3.6	3.6
Salt-soluble	1.3	1.3	1.6	1.6	1.7	1.5	1.4
Alcohol-soluble	6.9	5.6	6.3	5.6	7.6	5.2	6.7
Acetic acid-soluble	4.6	3.4	3.7	3.1	6.1	3.4	2.6
Residue	82.7	86.5	85.0	86.5	79.7	86.4	85.7
Recovery	94.9	97.4	97.4	97.5	92.6	97.6	96.0

It can be seen from Table 3 that the solubility distributions for the extracted tetraploids are quite different from those for the hexaploid parents. All except one of the soluble fractions from the three extracted tetraploids contained more material than the analogous fraction from the hexaploids. The exception was the amount of the salt-soluble fractions which was essentially the same for all the wheats studied. Of the four soluble fractions the alcohol-soluble fraction comprised the highest percentage of the original flour. Of the three varieties, the distribution for Tetraprelude was closest to that of its hexaploid parent. This seems to parallel the observation that this extracted tetraploid was similar to its hexaploid counterpart in breadmaking quality.

5. Protein Content of the Solubility Fractions

The protein contents (N x 5.7) of the various solubility fractions are given in Table 4. Nitrogen recoveries for the samples were above 96% and are considered satisfactory. In general, the protein contents of the soluble fractions from the extracted tetraploids were somewhat lower than those of the analogous fractions from the hexaploid parents. There were a number of minor deviations from this generalization. The water-soluble fractions contained from 45 to 58% protein while the alcohol-soluble fractions contained from 70 to 75% protein. The salt-soluble fractions were the highest in protein (76 - 78%) while the insoluble residue had the lowest protein content. The fact that the protein content for all of the soluble fractions was considerably less than 100% indicates that relatively large and variable amounts of non-protein

Table 4. Protein Contents of the Various Solubility Fractions

	<u>Water-Soluble</u>	<u>Salt-Soluble</u>	<u>Alcohol-Soluble</u>	<u>Acid-Soluble</u>	<u>Residue</u>
	%	%	%	%	%
Tetraprelude	51.3	77.0	74.5	70.3	6.8
Prelude	54.2	76.5	75.1	73.1	6.0
Tetrarescue	47.3	76.0	72.1	63.3	5.6
Rescue	57.6	77.9	70.5	69.6	5.3
Tetrathatcher	45.0	77.5	73.2	64.5	5.0
Thatcher	51.9	76.4	75.0	69.2	5.7
Stewart 63	47.9	75.7	69.8	58.8	4.2

material were extracted by the solvent used. Although carbohydrate analyses were not made in this study, a comparison of the data of Table 4 with those of Yong (81) suggests that most of the non-protein material is probably carbohydrate.

6. Distribution of Proteins in the Solubility Fractions

The distribution of proteins in the five fractions are given in Figs. 2 and 3. Figure 2 compares the results for the extracted tetraploid from Thatcher and Rescue with those for its hexaploid counterparts while Fig. 3 shows analogous results for Prelude. Both figures include results for Stewart 63, the durum wheat, for comparison purposes.

All seven species contained relatively small quantities of water (about 12%) and salt (about 8%) soluble proteins. Together these proteins made up approximately 20% of the flour proteins. There were no significant differences in the amounts of water-soluble and salt-soluble proteins among the seven wheats used in this investigation.

On the other hand, Tetrarescue and Tetrathatcher contained considerably more alcohol-soluble proteins, or "gliadin", and acetic acid-soluble protein, or "glutenin" and less insoluble protein than their hexaploid counterparts. In this respect, the extracted tetraploids of these two varieties were similar to the durum wheat Stewart 63. In contrast Tetraprelude and Prelude contained essentially the same amounts of protein in these three fractions.

It appears that removal of the D genome from the hexaploid to produce the extracted tetraploid wheat markedly affected the

Fig. 2. Distribution of proteins in the five solubility fractions for Tetrathatcher, Tetrarescue, Thatcher, Rescue and Stewart 63.

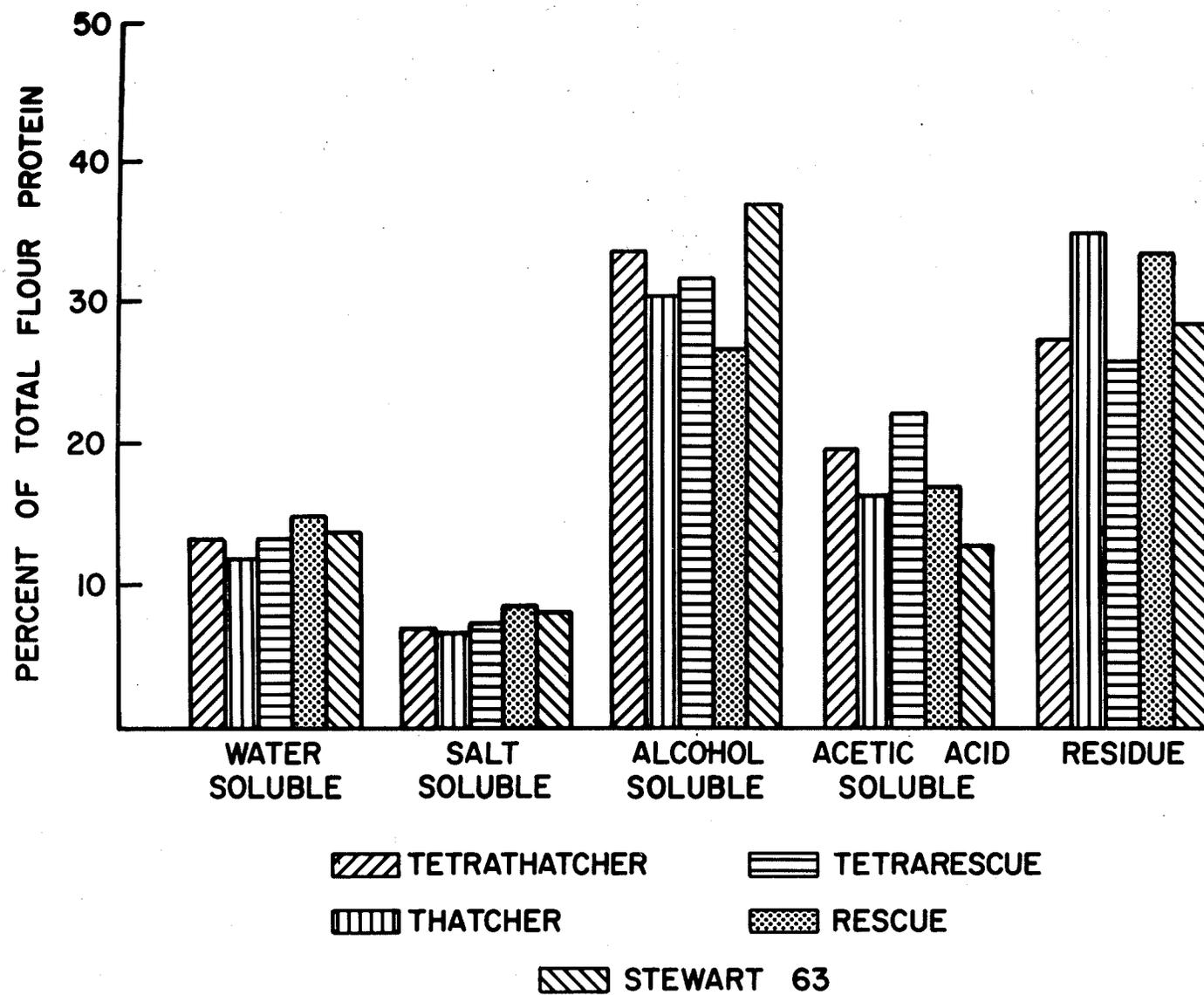
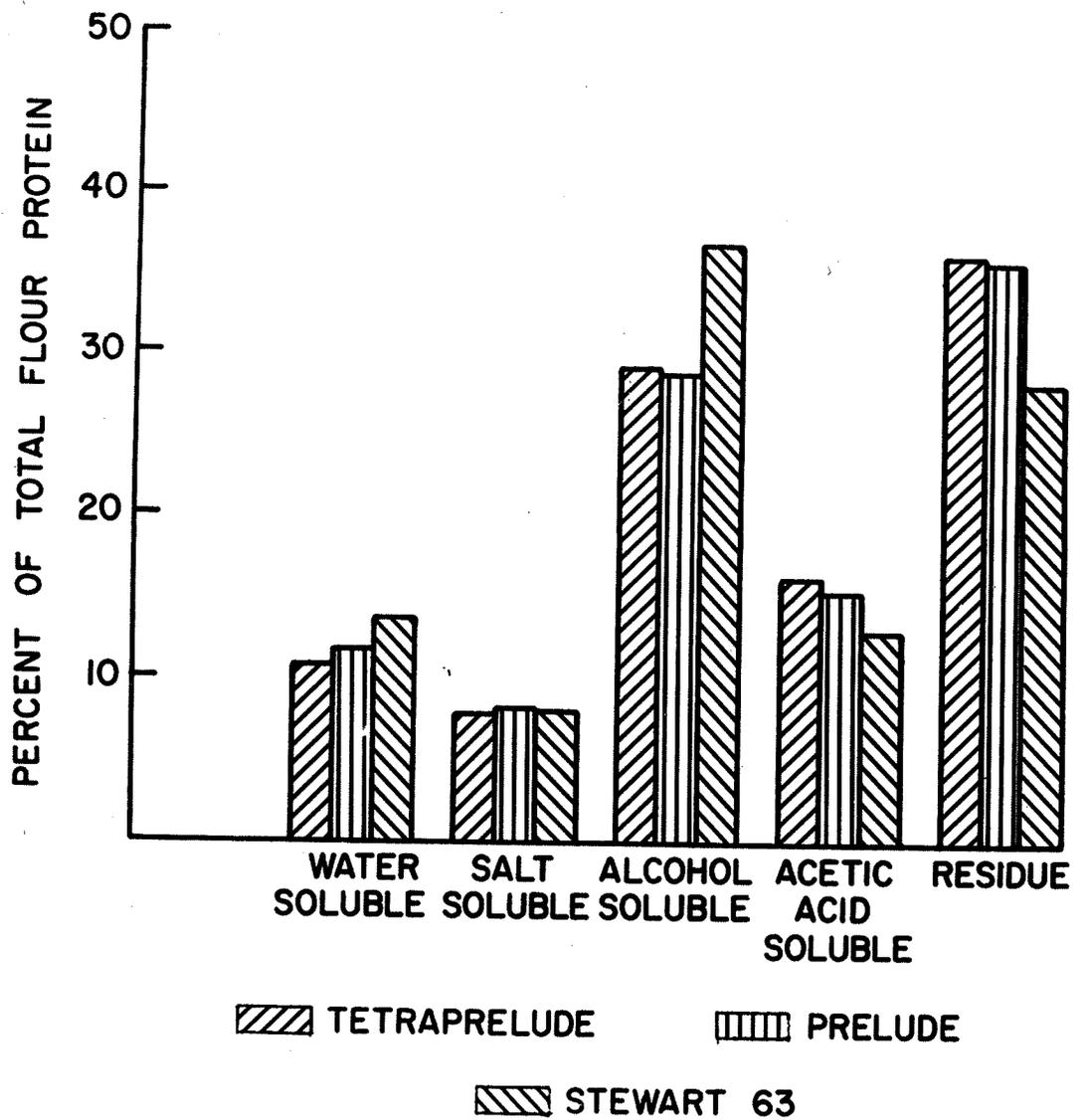


Fig. 3. Distribution of proteins in the five solubility fractions for Tetraprelude, Prelude and Stewart 63.



quantitative distribution of the proteins among solubility fractions in two of the three varieties. Tetraprelude, "the durum-type wheat with the high breadmaking quality" had essentially the same quantitative protein distribution as its hexaploid parent. These results suggest that breadmaking quality of wheats is directly related to the solubility composition of its proteins. However, since solubility of protein depends on chemical composition and structure, the present findings do not preclude the possibility that there might be qualitative differences within a particular solubility fraction that can affect the breadmaking quality. In the sections that follow, the proteins of various fractions will be examined to determine if there are any qualitative differences among them.

7. Amino Acid Composition

The amino acid compositions of the flour and of the various solubility groups were determined on a Beckman-Spinco Model 120 amino acid analyzer. In all analyses, nitrogen recoveries following chromatography of the hydrolyzates was over 95%. No corrections were applied for the loss of threonine or serine during hydrolysis. Cysteine, cystine, and tryptophan contents were not determined. The amino acid contents will be expressed in g. of amino acid per 100 g. protein.

Amino acid compositions of the flours from the three extracted tetraploids, their parents and the durum wheat variety Stewart 63 are given in Table 5. Comparison of the data for each extracted tetraploid and its hexaploid counterpart, shows that the compositions

are essentially the same. No significant differences in amino acid composition were found in all the seven flours analyzed. These results are in general agreement with the findings of Tkachuk (56) who reported that hard red spring wheat flours of good and poor baking quality as well as flours from four different classes of Canadian wheat showed little variation in their amino acid compositions.

The amino acid compositions of the various solubility fractions obtained from the seven flours are given in Tables 6, 7, 8 and 9. The compositions of the four protein fractions for a single variety or species are quite different. However, the compositions of any one fraction, from different varieties and species were essentially the same. There was no significant differences in amino acid composition in any of the soluble protein fractions from wheats of good and poor baking quality. Although, the tetraploids of Rescue and Thatcher had significantly different solubility distribution patterns than their corresponding hexaploids (Fig. 2), this difference was not reflected by the amino acid composition data. Presumably, the differences in protein composition were not large enough to show significant differences in amino acid composition of the total endosperm.

Particular solubility fractions were characterized by relatively high contents of certain amino acids. The water and salt-soluble fractions were relatively rich in basic amino acids and poor in glutamic acid (Tables 6 and 7). The alcohol-soluble fraction was characterized by high glutamic acid and proline contents

Table 5. Amino Acid Compositions of the Flours

(g. of amino acid per 100 g. of protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	2.5	2.3	2.7	2.7	2.4	2.6	2.4
Histidine	2.5	2.3	2.5	2.5	2.3	2.7	2.2
Ammonia	4.4	4.6	4.1	4.2	4.1	4.5	3.7
Arginine	4.7	4.8	4.5	4.8	4.4	4.7	4.3
Aspartic	5.8	5.4	4.9	5.2	5.7	5.5	5.0
Threonine	3.2	3.1	3.1	3.2	3.1	3.1	2.9
Serine	5.1	5.4	4.9	5.3	5.8	5.5	4.8
Glutamic	43.6	46.5	42.8	44.4	43.8	45.5	48.8
Proline	13.9	13.6	13.0	13.5	13.6	14.1	13.2
Glycine	4.2	4.4	4.2	4.6	4.2	4.4	4.1
Alanine	3.5	3.7	3.5	3.7	3.8	3.9	3.5
Valine	5.1	5.1	4.6	4.8	5.1	5.1	4.7
Methionine	1.4	1.4	1.3	1.4	1.5	1.4	1.4
Isoleucine	3.9	4.3	4.0	4.0	4.6	4.2	4.5
Leucine	8.5	8.4	7.8	8.0	8.7	8.3	8.8
Tyrosine	3.0	3.0	3.0	3.4	2.9	2.9	3.1
Phenylalanine	6.3	6.5	5.7	6.0	6.0	6.0	6.5

Table 6. Amino Acid Compositions of Water-Soluble Proteins

(g. amino acid per 100 g. of protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	4.8	4.7	5.1	5.1	5.2	4.6	4.7
Histidine	2.6	2.3	2.7	2.7	2.8	2.4	2.3
Ammonia	2.9	2.8	2.4	2.7	2.7	2.3	2.4
Arginine	5.0	5.2	6.2	6.4	6.2	6.0	5.6
Aspartic	8.2	7.8	8.8	8.3	8.2	8.3	8.9
Threonine	4.2	3.9	4.3	4.1	4.6	4.2	4.4
Serine	5.3	4.9	5.5	5.2	5.3	5.1	5.1
Glutamic	30.2	27.2	27.2	28.2	29.7	26.7	26.8
Proline	12.3	11.4	10.7	11.8	11.3	11.0	10.4
Glycine	5.0	4.9	5.5	5.1	5.5	5.0	5.3
Alanine	5.6	5.4	6.3	6.3	6.3	6.0	5.9
Valine	5.8	5.8	6.4	5.8	6.0	5.8	6.1
Methionine	2.1	1.9	2.3	2.6	2.7	2.3	2.5
Isoleucine	4.1	3.8	4.2	4.3	4.1	4.0	4.2
Leucine	8.3	8.5	8.7	9.4	9.0	8.5	8.7
Tyrosine	3.9	3.9	3.9	4.1	3.7	3.6	4.0
Phenylalanine	5.9	6.0	5.2	5.6	5.4	5.0	5.0

Table 7. Amino Acid Compositions of Salt-Soluble Proteins
(g. of amino acid per 100 g. of protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	7.3	6.3	6.2	6.4	6.1	6.7	5.5
Histidine	3.6	3.1	3.3	3.3	3.1	3.1	3.2
Ammonia	1.9	1.7	1.7	1.7	1.7	1.7	1.8
Arginine	8.6	8.2	10.3	9.4	9.5	9.8	9.6
Aspartic	7.4	9.3	7.5	7.9	8.4	7.4	6.9
Threonine	4.0	4.6	3.6	3.9	4.1	3.9	3.6
Serine	5.2	5.6	4.7	4.7	5.3	4.8	5.0
Glutamic	17.4	20.1	17.9	17.8	19.3	16.9	19.9
Proline	4.8	5.7	5.0	5.2	5.5	5.0	5.3
Glycine	6.7	5.9	6.2	5.5	6.2	5.6	5.1
Alanine	7.0	6.0	5.6	5.5	5.9	5.3	4.8
Valine	6.6	6.4	6.2	6.0	6.1	6.2	4.9
Methionine	1.3	1.5	2.0	2.0	2.2	2.3	1.4
Isoleucine	4.3	4.6	4.0	4.1	4.5	4.6	4.0
Leucine	8.4	8.6	7.5	8.0	8.5	8.2	7.3
Tyrosine	3.2	3.2	3.7	3.6	3.1	3.5	3.1
Phenylalanine	4.6	4.8	5.0	4.5	4.7	5.2	4.6

Table 8. Amino Acid Compositions of Acid-Soluble Proteins

(g. of amino acid per 100 g. protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	1.7	1.7	1.5	1.4	1.5	1.4	1.6
Histidine	2.1	2.3	2.3	2.1	2.1	2.3	2.3
Ammonia	4.3	4.6	5.2	4.9	4.4	5.0	5.4
Arginine	3.5	3.3	3.8	3.5	3.2	3.3	3.5
Aspartic	3.0	2.9	3.2	2.9	3.0	2.9	3.1
Threonine	2.3	2.7	3.1	2.7	2.9	2.9	2.7
Serine	5.4	5.1	5.6	5.0	5.1	5.6	5.4
Glutamic	50.2	55.1	58.8	55.1	49.6	54.5	58.5
Proline	14.4	15.6	15.9	15.0	12.9	15.0	16.0
Glycine	5.4	4.5	5.3	4.7	4.6	4.3	4.4
Alanine	2.8	2.4	2.7	2.3	2.6	2.5	2.7
Valine	3.7	3.8	4.1	4.6	3.5	4.1	4.4
Methionine	1.5	1.3	1.7	1.5	1.2	1.5	1.2
Isoleucine	3.6	4.1	4.4	3.8	3.9	4.7	4.9
Leucine	7.4	7.9	8.4	7.2	7.7	8.3	8.5
Tyrosine	4.5	4.3	5.0	4.4	3.6	3.3	4.5
Phenylalanine	6.1	7.3	6.7	7.1	5.8	7.7	8.4

Table 9. Amino Acid Compositions of Alcohol-Soluble Proteins
(g. of amino acid per 100 g. of protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	0.7	0.6	0.6	0.6	0.6	0.6	0.7
Histidine	2.0	2.1	2.3	2.2	2.1	2.3	2.0
Ammonia	4.3	4.6	4.5	4.9	4.3	5.1	5.0
Arginine	2.4	2.4	2.7	2.4	2.5	2.7	2.8
Aspartic	2.7	2.8	2.9	3.0	2.9	3.0	3.3
Threonine	2.0	2.0	2.0	2.0	2.1	2.3	1.8
Serine	4.4	4.8	4.7	4.2	4.4	5.1	4.2
Glutamic	54.1	57.3	55.8	55.8	54.0	56.4	59.6
Proline	17.9	19.4	17.2	18.0	16.8	17.5	18.9
Glycine	1.5	1.6	1.6	1.7	1.6	1.8	1.9
Alanine	2.1	2.1	2.3	2.3	2.3	2.4	2.4
Valine	3.8	4.1	4.1	4.4	4.0	4.6	4.7
Methionine	1.1	1.1	1.2	1.3	1.4	1.3	1.8
Isoleucine	4.6	4.6	4.7	4.6	4.5	4.7	5.3
Leucine	8.1	8.2	8.2	8.3	7.9	7.8	8.7
Tyrosine	2.5	2.9	2.8	3.3	2.4	2.8	3.8
Phenylalanine	7.4	7.6	6.7	7.0	7.3	7.2	8.3

and low lysine content (Table 8).

The composition of the alcohol-soluble fractions was, in general, quite similar to that of the acid-soluble fractions. Similar results were obtained by Ewart (60). Some minor differences do exist between these two fractions. The alcohol-soluble protein fraction is richer in proline than the acid-soluble fraction. On the other hand, the acetic acid-soluble fraction had much higher glycine, lysine, and tyrosine contents.

8. Starch-Gel and Disc Electrophoresis Results

(i) Introduction - Both disc and starch-gel electrophoresis were used to compare the proteins of the various solubility groups, as well as the protein extracts obtained from single seeds of the other cereal species studied. Disc electrophoresis has greater resolving power than the starch-gel electrophoresis technique. Another advantage of disc electrophoresis is its ability to yield rapid and reproducible separations of the proteins studied. The main difficulties encountered with starch-gel electrophoresis were the lack of reproducibility and the overall inconvenience of the technique. Accordingly, disc electrophoresis was used for most of this study. However, starch-gel electrophoresis results were included in some cases for comparison.

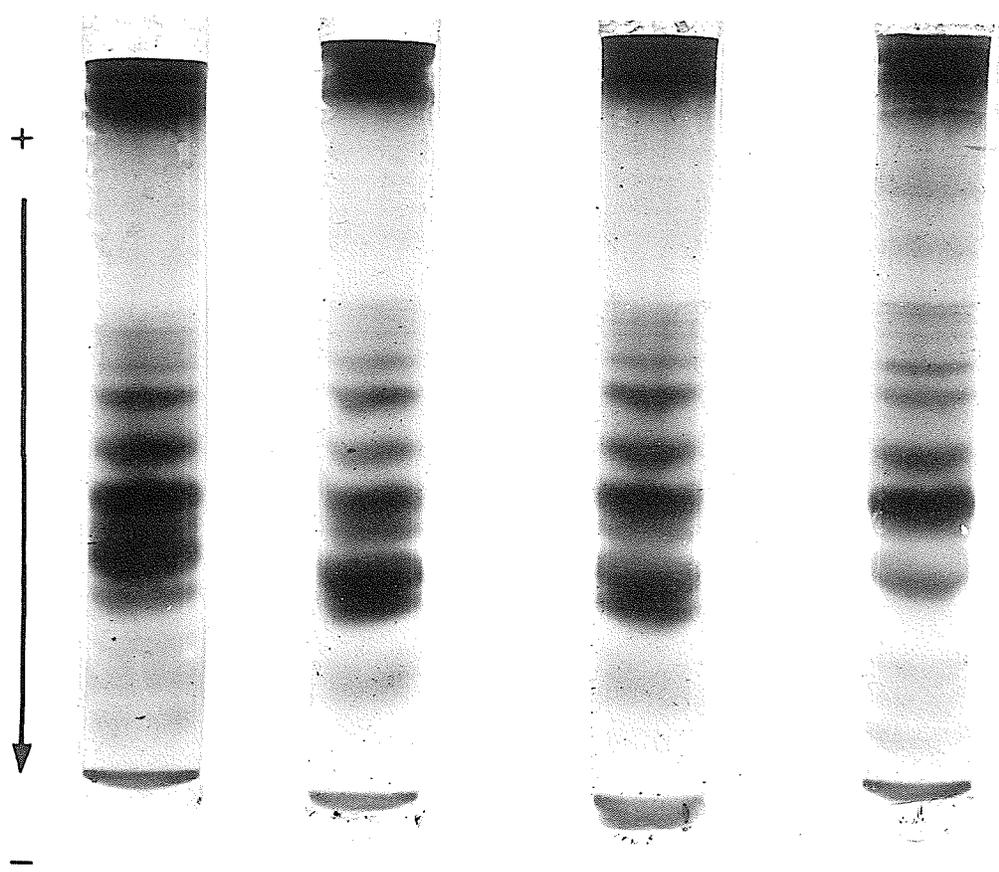
The proteins of the extracted tetraploids, their parents as well as the other cereal species studied were compared on the basis of their patterns after electrophoresis. Relative mobility of the protein components identified as individual bands was used as the

criterion of identity or homology of the proteins in the same fraction or extract from different varieties or species. This procedure is similar to that employed by Johnson et al (63) in the analysis of genome and species relationships in wheat. Proteins which had the same electrophoretic mobility were considered to be identical or very similar in physical and chemical properties. To confirm the electrophoretic identity of the protein components of each extracted tetraploid and its parent, protein mixtures made up of equal parts of the same fractions from the two related species were examined by electrophoresis under identical conditions. Identical proteins from the two species in the mixture will appear as a single band.

(ii) Water-Soluble Proteins - Figures 4 - 6 show the disc electrophoresis patterns of the water-soluble proteins or the "albumins" of the three extracted tetraploids, their parents, Stewart 63 and mixtures of the fractions from each tetraploid and its parent. The water-soluble proteins are characterized by intermediate and fast moving protein bands with relative mobilities (R_f) from 0.35 to 0.90. The patterns for each extracted tetraploid was essentially the same as that for its hexaploid counterpart. This was confirmed by comparing patterns for the tetraploid, its parent and their mixture. Detailed examination of the patterns showed that the water-soluble proteins from hexaploid Rescue might contain one very minor protein band (R_f of 0.75) that is not present in the pattern for the fraction of its extracted tetraploid. On the basis of these results it appears that the removal of the

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Fig. 4. Disc electrophoretic patterns for the albumins from Tetraprelude (4xP), Prelude (6xP), Stewart 63 (ST63) and a mixture (M) of the albumins from Tetraprelude and Prelude.



4xP

6xP

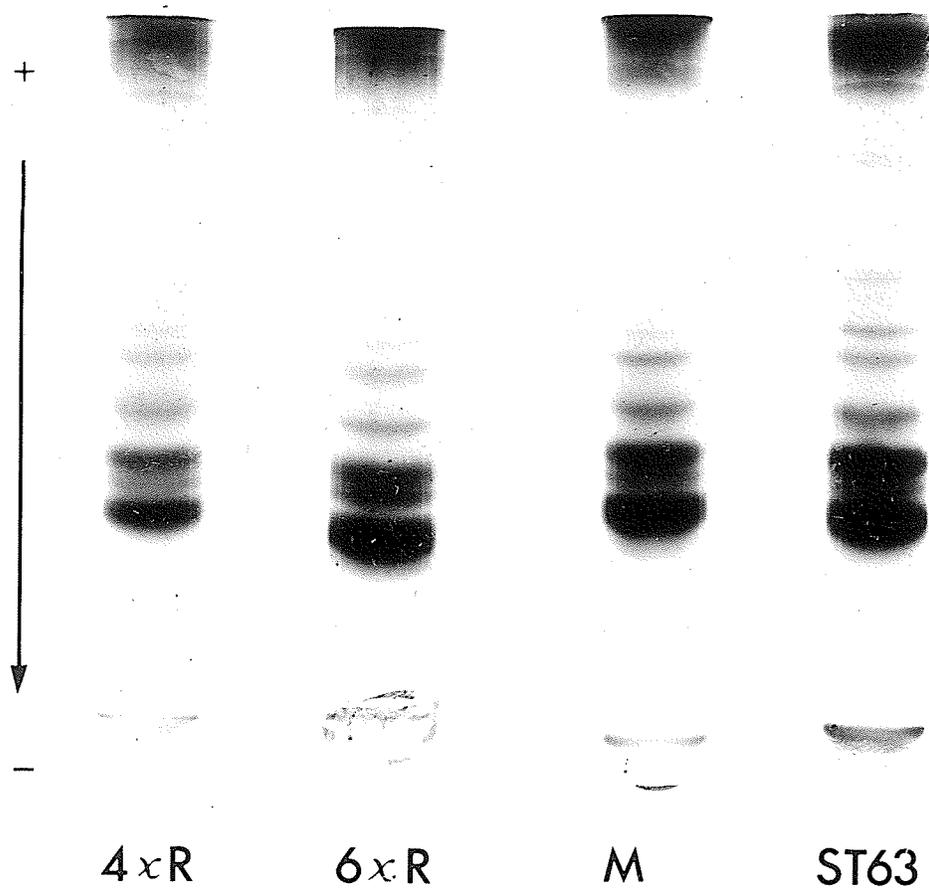
M

ST63

ALBUMINS

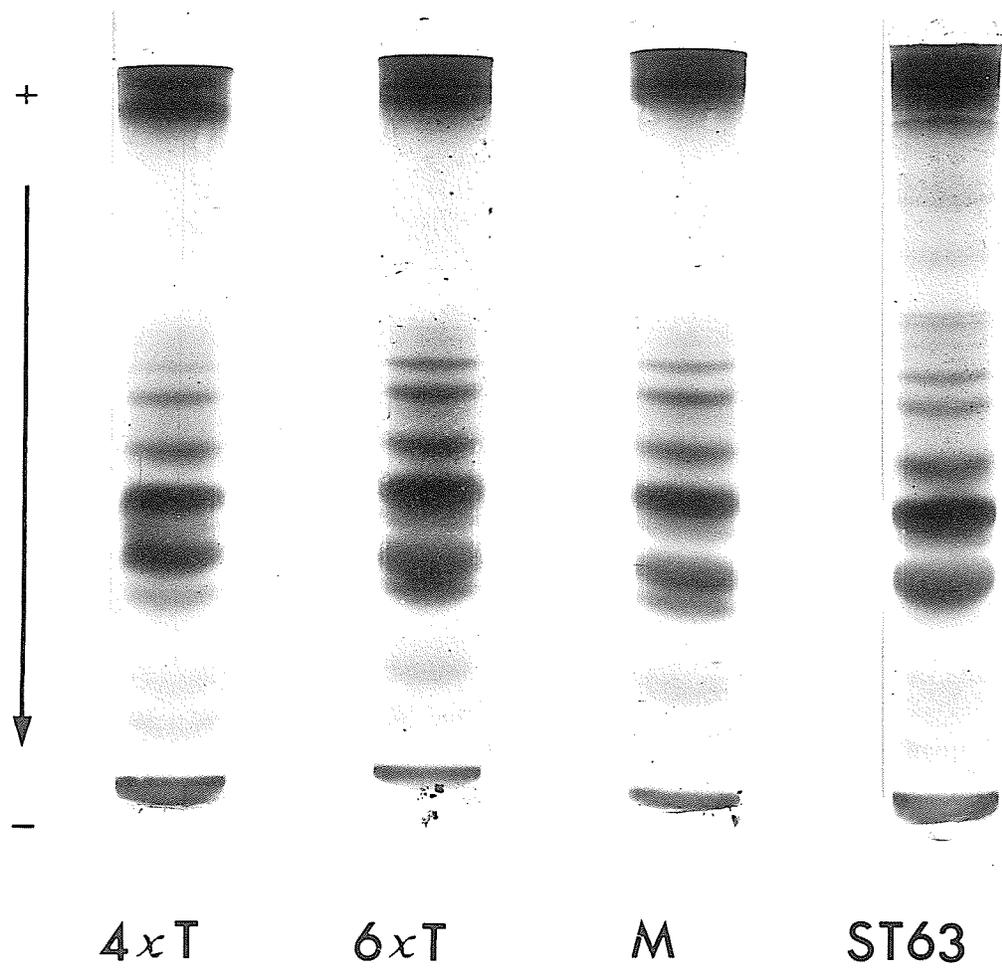
2

Fig. 5. Disc electrophoretic patterns for the albumins from Tetrarescue (4xR), Rescue (6xR), Stewart 63 (ST63) and a mixture (M) of albumins from Tetrarescue and Rescue.



ALBUMINS

Fig. 6. Disc electrophoretic patterns for the albumins from Tetrathatcher (4xT), Thatcher (6xT), Stewart 63 (ST63) and a mixture (M) of albumins from Tetrathatcher and Thatcher.



4xT

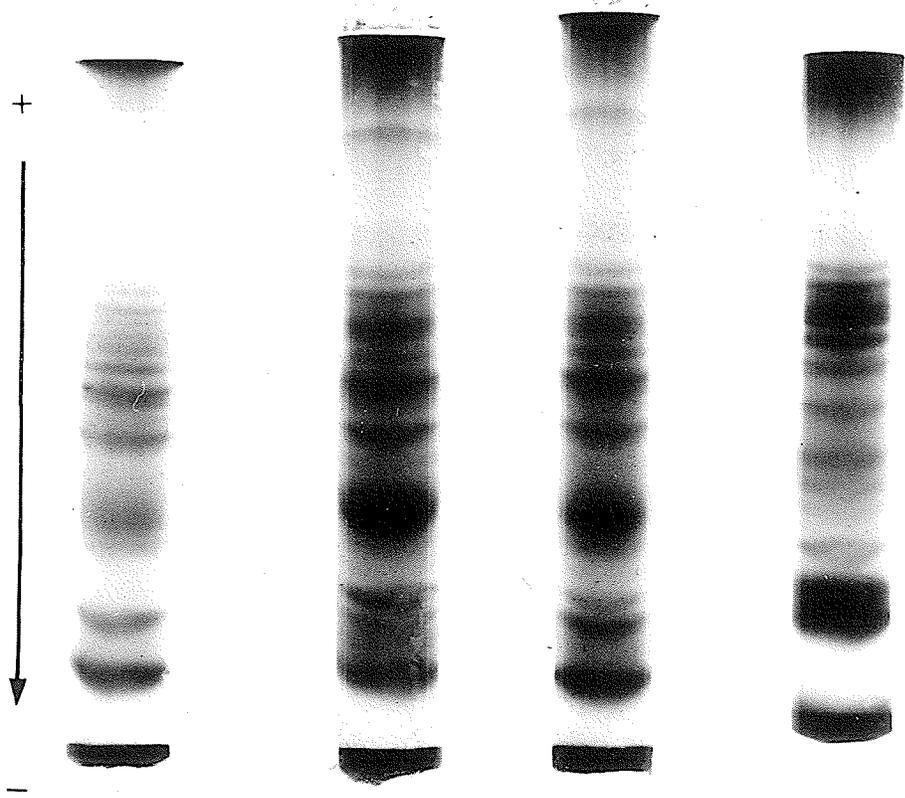
6xT

M

ST63

ALBUMINS

Fig. 7. Disc electrophoretic patterns for the globulins from Tetraprelude (4xP), Prelude (6xP), Stewart 63 (ST63) and a mixture (M) of globulins from Tetraprelude and Prelude.



4xP

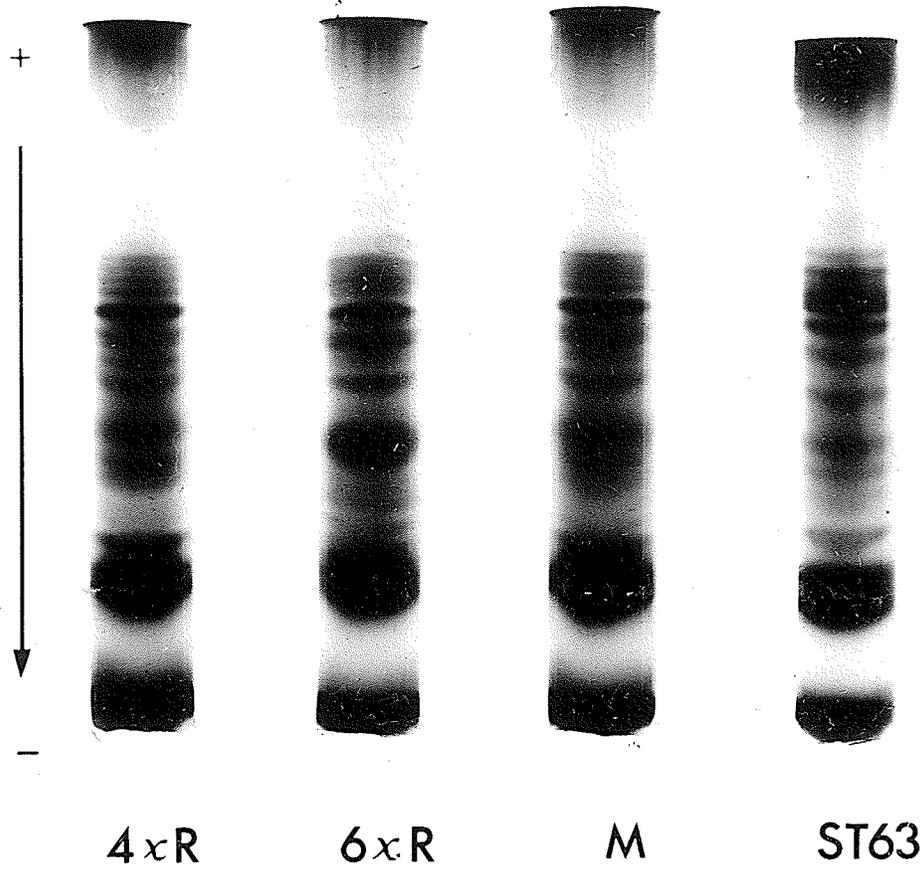
6xP

M

ST63

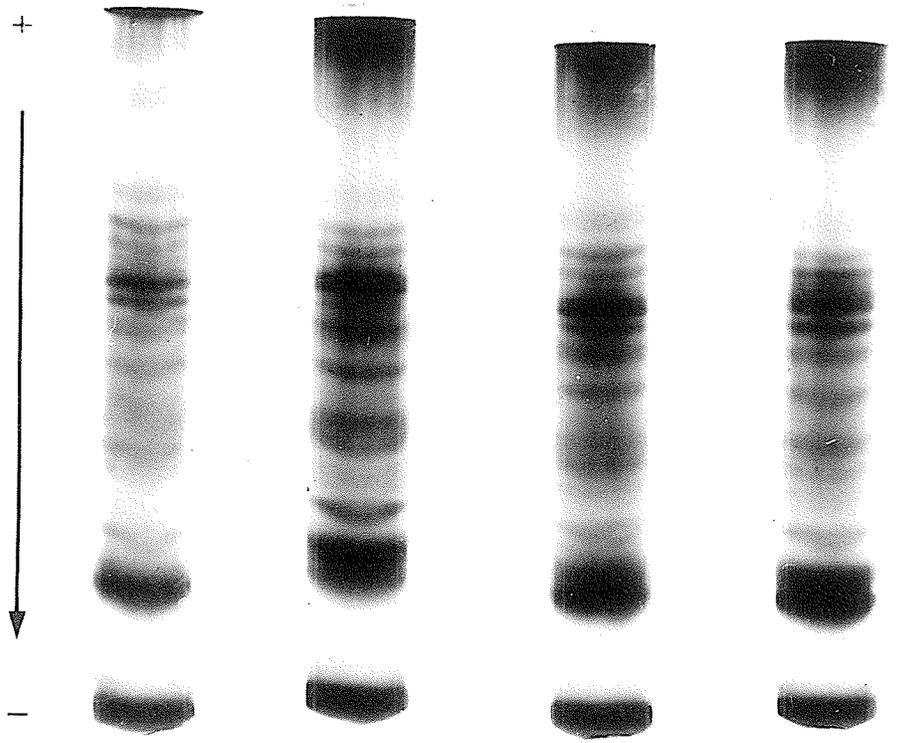
GLOBULINS

Fig. 8. Disc electrophoretic patterns for the globulins from Tetrarescue (4xR), Rescue (6xR), Stewart 63 (ST63) and a mixture (M) of globulins from Tetrarescue and Rescue.



GLOBULINS

Fig. 9. Disc electrophoretic patterns for the globulins from Tetrathatcher (4xT), Thatcher (6xT), Stewart 63 (ST63) and a mixture (M) of globulins from Tetrathatcher and Thatcher.



4xT

6xT

M

ST63

GLOBULINS

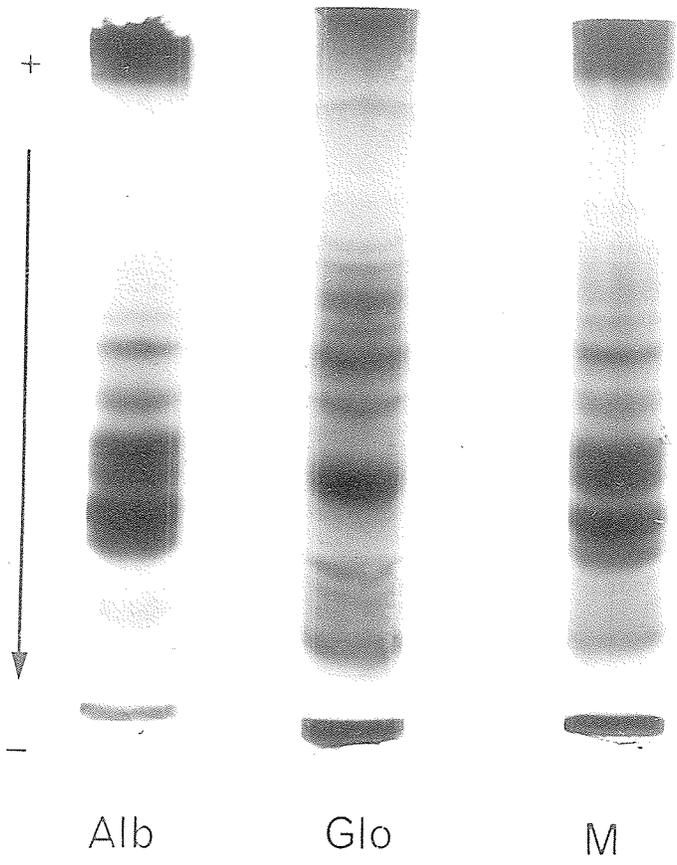
D genome from the three hexaploid wheats did not alter the qualitative composition of the water-soluble proteins, except perhaps the deletion of one minor protein band in the variety Rescue.

The three hexaploid wheats studied were very homogenous with respect to the water-soluble proteins. Their protein patterns comprised at least 10 detectible bands. Four major protein bands appeared in the R_f region from 0.56 to 0.70. It was not possible to ascertain by the electrophoretic technique used whether any of these 4 major bands contained more than one protein. A dark band at the origin and a few light bands in the R_f region from 0 to 0.35 were observed for all the wheats examined. These appear to be due to contamination with the alcohol and acetic acid-soluble proteins extracted in the water-soluble fraction.

Comparison of patterns for the water-soluble proteins of the durum and the three bread wheat varieties showed that all of the water-soluble proteins of the durum wheat were present in the three hexaploid wheats. However, the hexaploid wheats had one minor additional fast moving protein band at R_f 0.75 which was not present in the durum wheat. Two of the three extracted tetraploids (Tetraprelude and Tetrathatcher) retained this minor, fast moving band. Accordingly, it appears that in the variety Rescue this band was contributed by the D genome, while in Thatcher and Prelude, the genes that control its synthesis are also present in the A and B genomes.

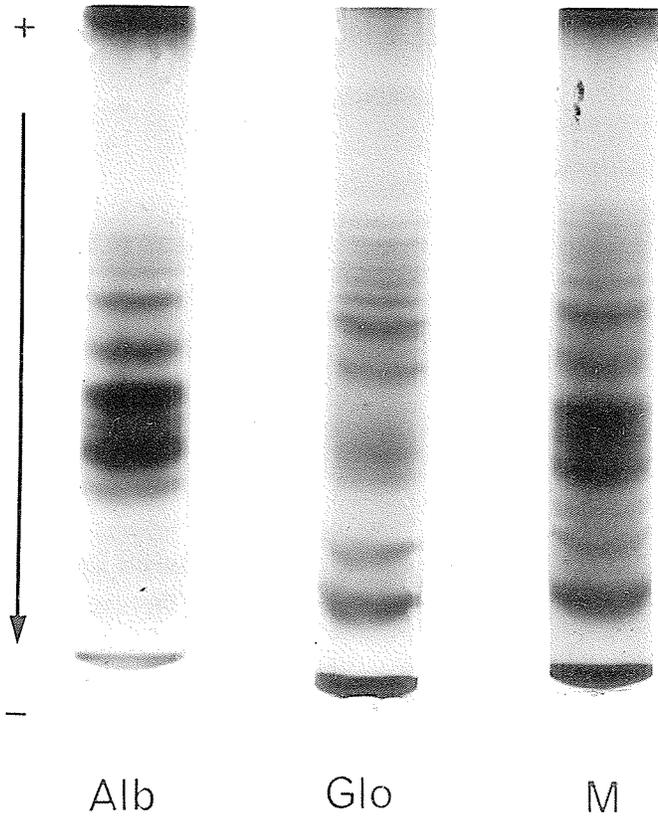
(iii) Salt-Soluble Proteins - The disc electrophoresis patterns for the salt-soluble proteins or "globulins" of the three

Fig. 10. Disc electrophoretic patterns for the albumins (Alb),
the globulins (Glo) and their mixture (M) from Prelude.



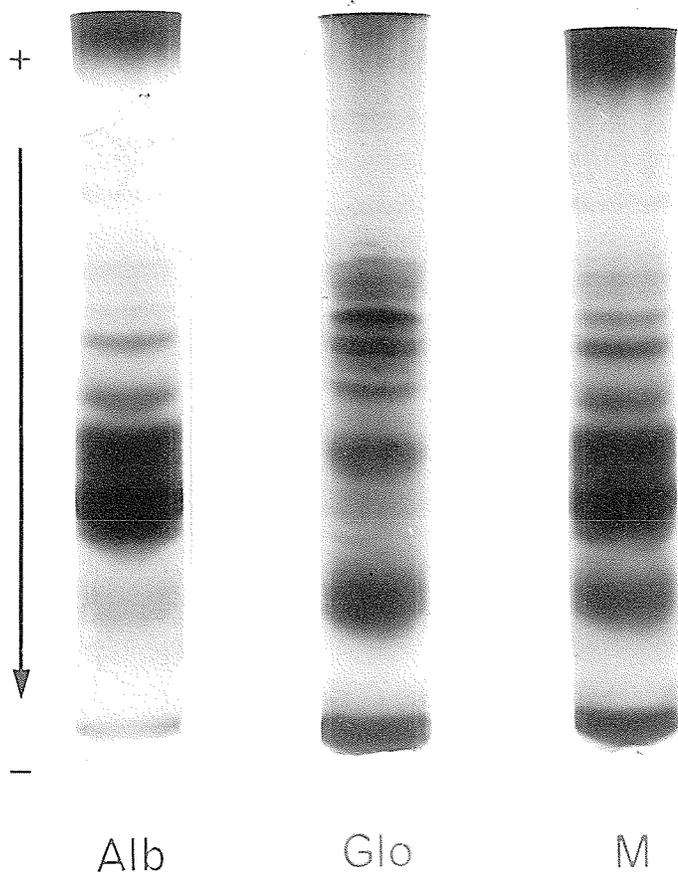
PRELUDE

Fig. 11. Disc electrophoretic patterns for the albumins (Alb), the globulins (Glo) and their mixture (M) from Tetra-
prelude.



TETRAPRELUDE

Fig. 12. Disc electrophoretic patterns for the albumins (Alb),
the globulins (Glo) and their mixture (M) from Rescue.



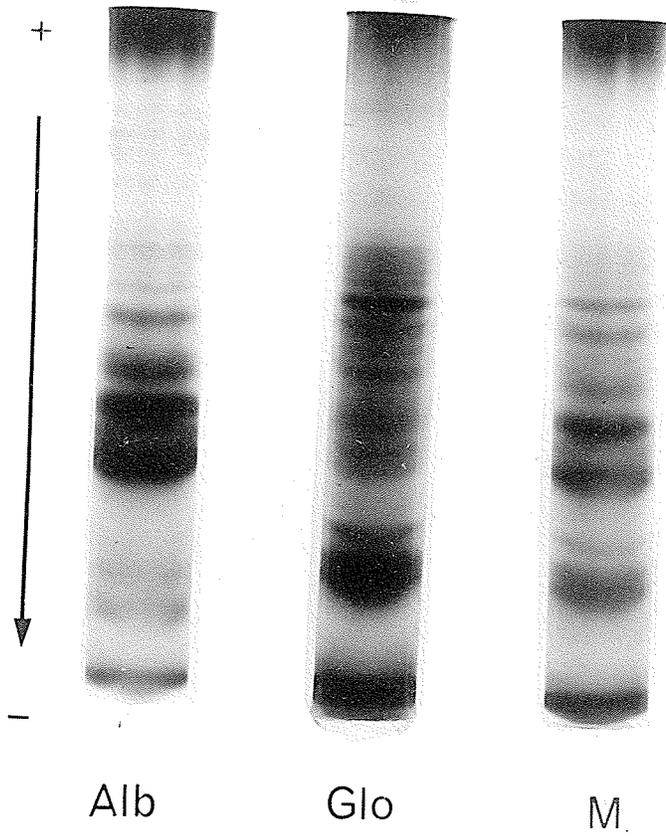
Alb

Glo

M

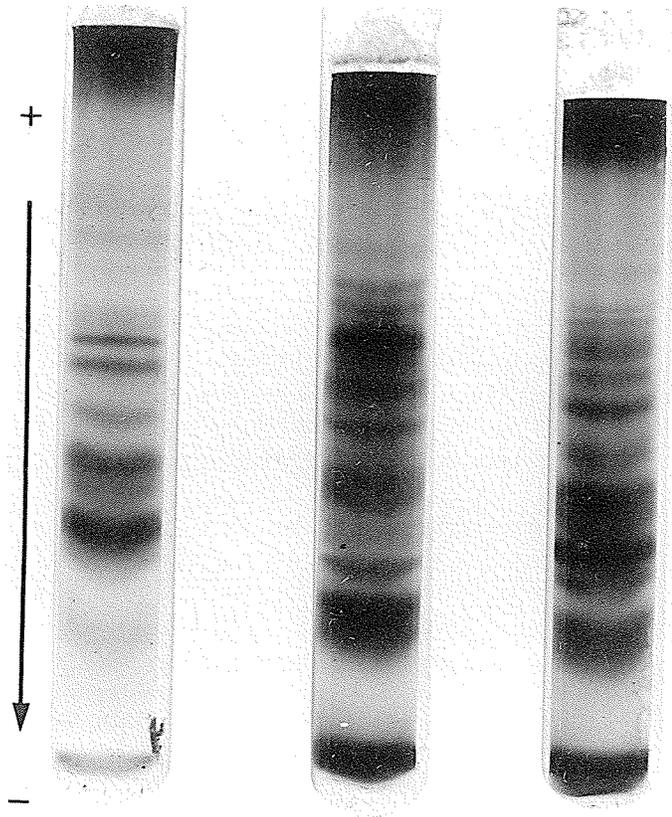
RESCUE

Fig. 13. Disc electrophoretic patterns for the albumins (Alb), the globulins (Glo) and their mixture (M) from Tetra-rescue.



TETRA RESCUE

Fig. 14. Disc electrophoretic patterns for the albumins (Alb),
the globulins (Glo) and their mixture (M) from Thatcher.



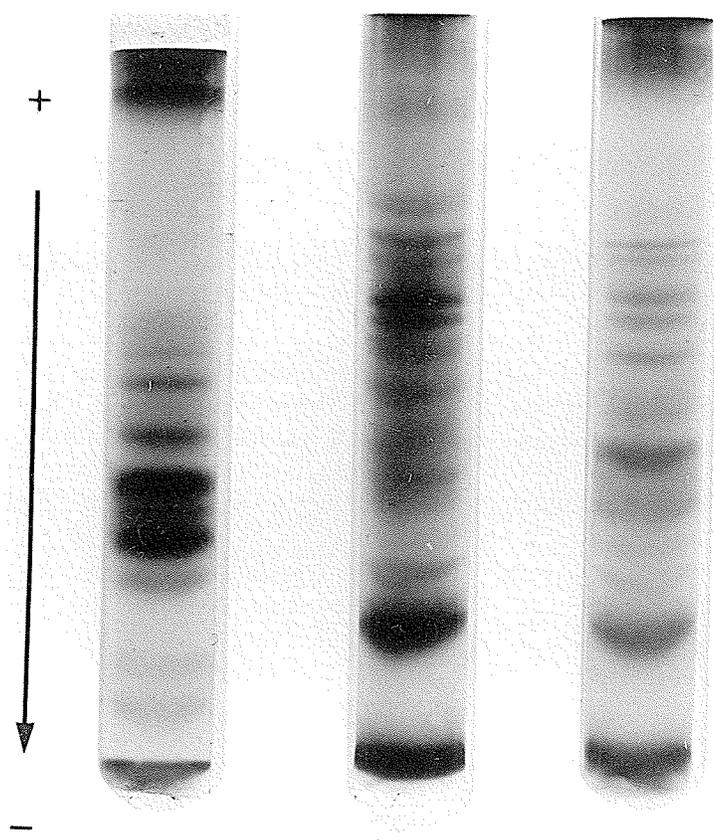
Alb

Glo

M

THATCHER

Fig. 15. Disc electrophoretic patterns for the albumins (Alb), the globulins (Glo) and their mixture (M) from Tetra-thatcher.



Alb

Glo

M

TETRATHATCHER

extracted tetraploids, their parents, durum wheat (Stewart 63) and mixtures of extracts of each extracted tetraploid and its parent are shown in Figures 7 - 9. The proteins components of this solubility group migrated in the R_f region from 0.3 to 0.9. The patterns for the extracted tetraploids and their parents are essentially the same. However, detailed examination of the patterns showed a number of relatively minor differences among the varieties studied. The pattern for the durum wheat was found to be quite similar to that of the other species studied. Since all of the salt-soluble protein bands present in each hexaploid were represented in the pattern of its extracted tetraploid, it appears that the genes that control the synthesis of the different salt-soluble proteins in bread wheats are in the A and B genomes.

Of all of the soluble protein fractions, the salt-soluble fraction contained the largest number of detectible bands. As many as 15 protein bands could be readily detected. Generally, the bands were sharp and intensively stained. The major band had the highest mobility and appeared to be a doublet. All extracts showed a dark band at the origin. Presumably, these could be high molecular weight globulins which did not migrate into the gel. A globulin protein with a molecular weight of about 200,000 was isolated from wheat flour by Danielson (37). Molecules of this size would not migrate into the gel.

Although, the salt-soluble proteins migrated with the same mobility as the water-soluble proteins, distinct differences were

Fig. 16. Disc electrophoretic patterns for the gliadins from Tetraprelude (4xP), Prelude (6xP), Stewart 63 (ST63) and a mixture (M) of the gliadins from Tetraprelude and Prelude.

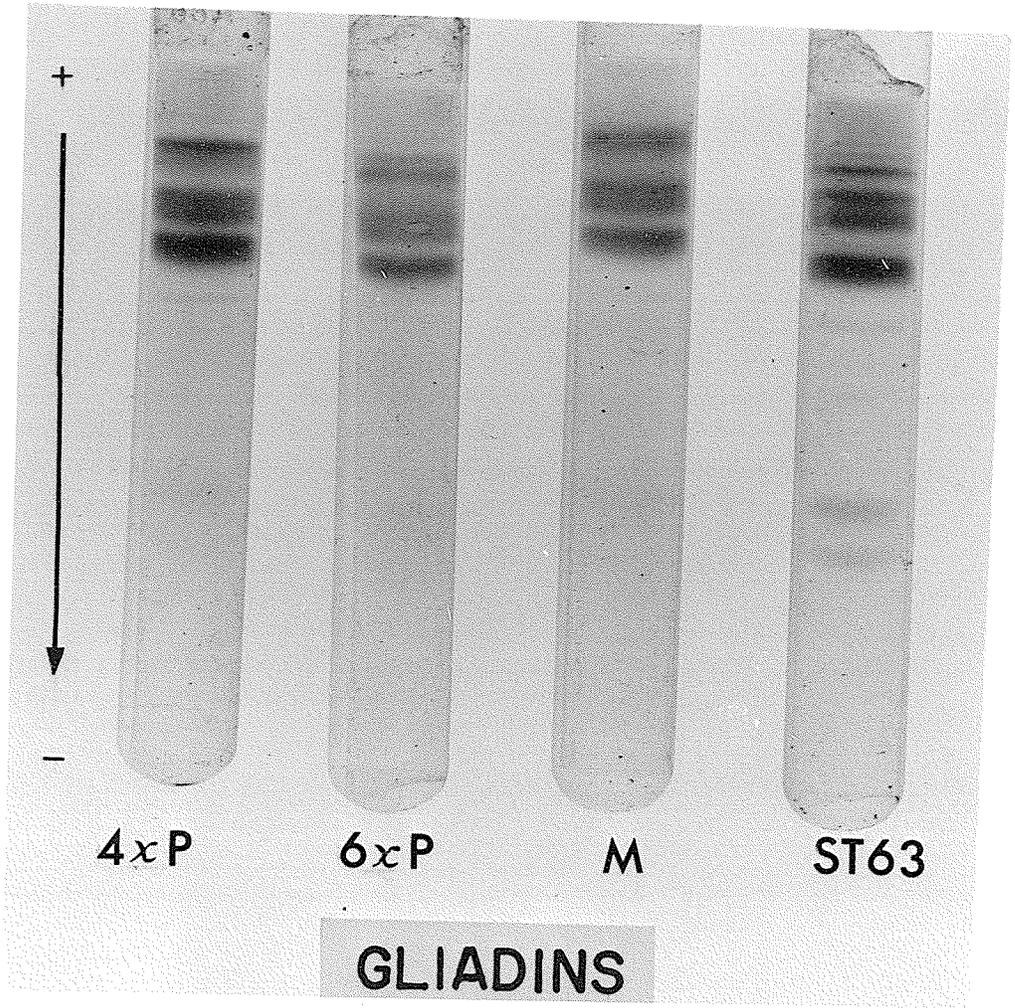
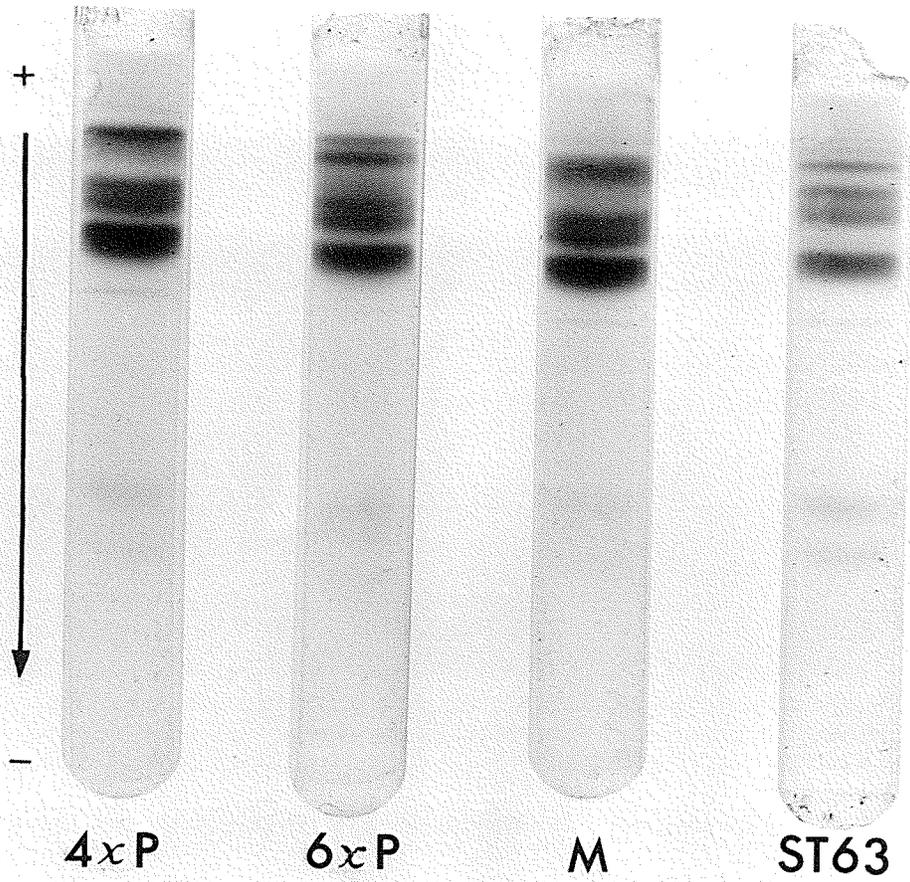


Fig. 17. Disc electrophoretic patterns for the gliadins from Tetraprelude (4xP) and Prelude (6xP) (2 times the concentration of Fig. 16), Stewart 63 (ST63) and a mixture (M) of the gliadins from Tetraprelude and Prelude.



GLIADINS

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Fig. 18. Disc electrophoretic patterns for the gliadins from Tetraprelude (4xP) and Prelude (6xP) (3 times the concentration of Fig. 16), Stewart 63 (ST63) and a mixture (M) of the gliadins from Tetraprelude and Prelude.

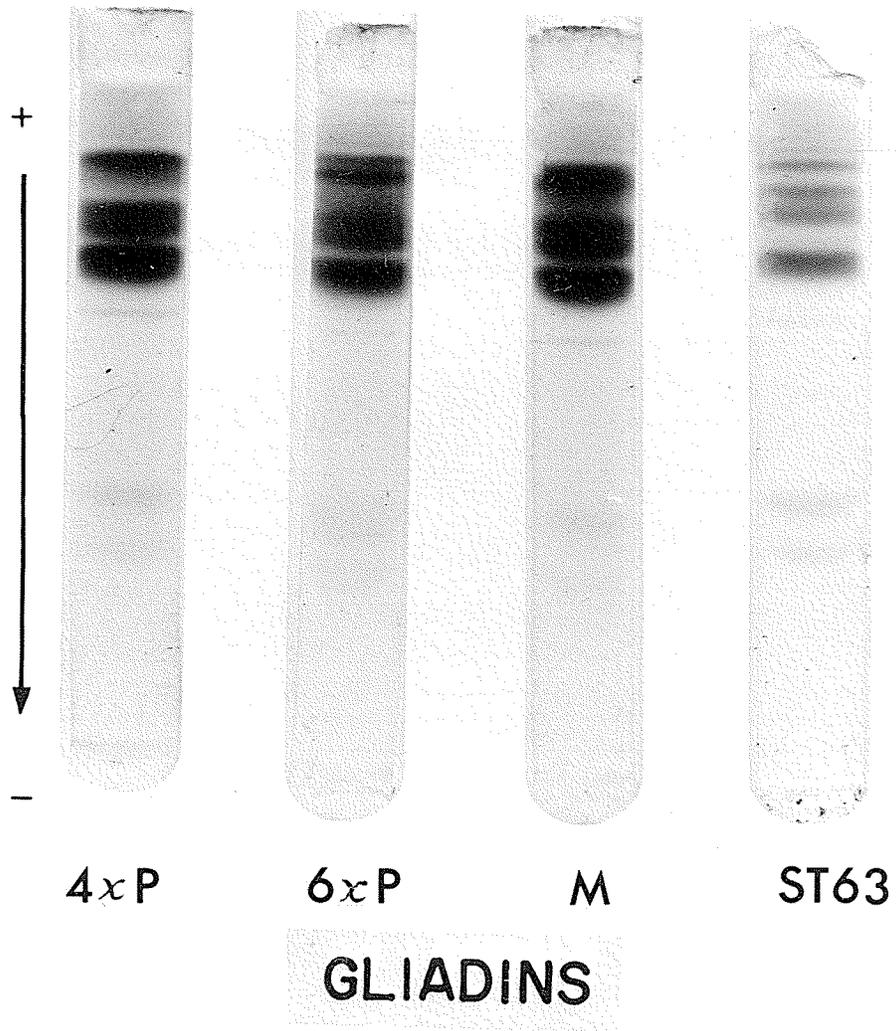
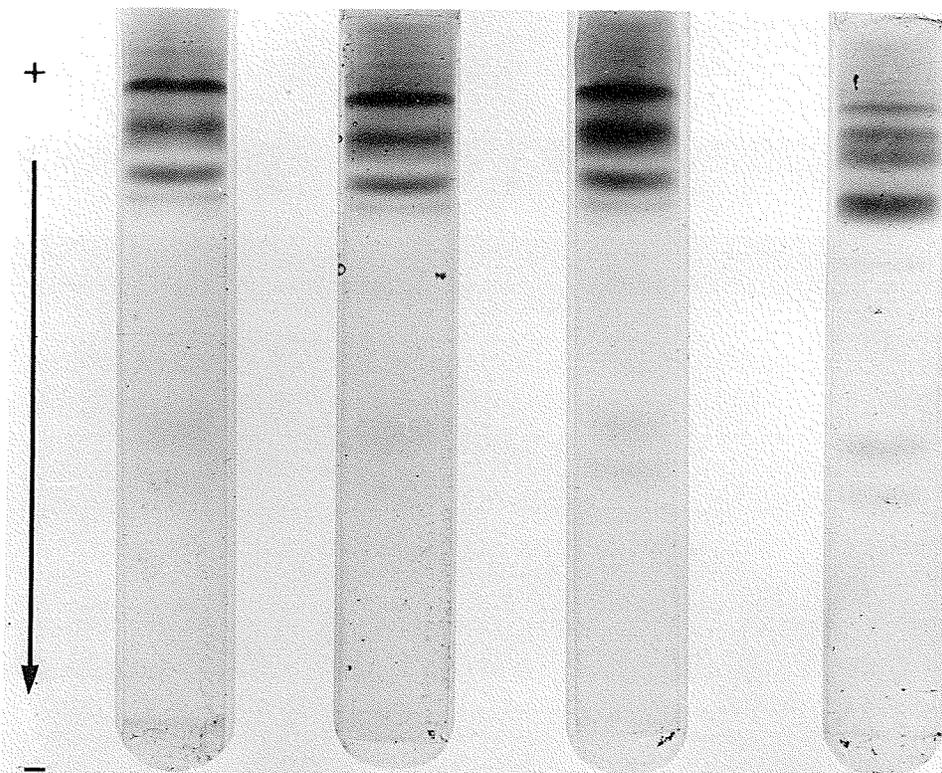


Fig. 19. Disc electrophoretic patterns for the gliadins from Tetrarescue (4xR), Rescue (6xR), Stewart 63 (ST63) and a mixture (M) of the gliadins from Tetrarescue and Rescue.



4xR

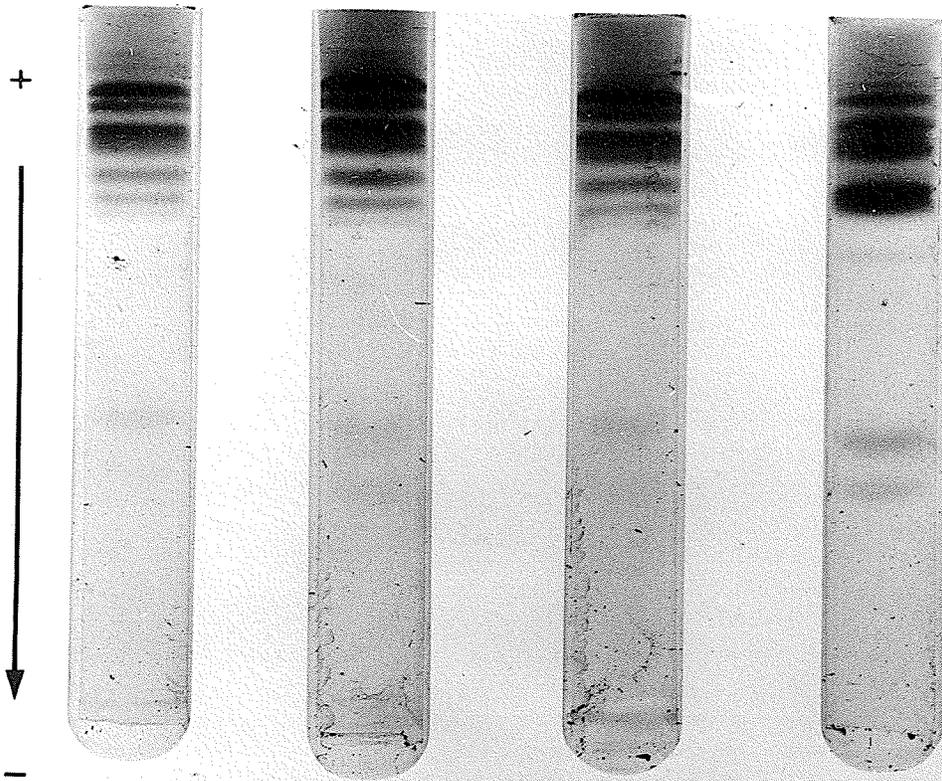
6xR

M

ST63

GLIADINS

Fig. 20. Disc electrophoretic patterns for the gliadins from Tetrathatcher (4xT), Thatcher (6xT), Stewart 63 (ST63) and a mixture (M) of the gliadins from Tetrathatcher and Thatcher.



4xT

6xT

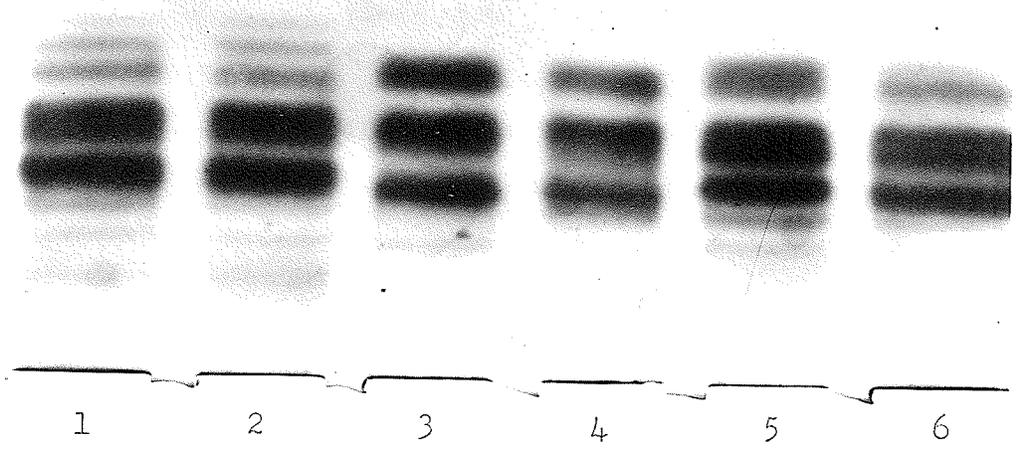
M

ST63

GLIADINS

Fig. 21. Starch-gel patterns for gliadins from the three extracted Tetraploids and their parents.

1. Tetrathatcher, 2. Thatcher, 3. Tetraprelude,
4. Prelude, 5. Tetrarescue, 6. Rescue.



observed in the patterns for the two solubility groups. However, there is a possibility that some of the proteins in the salt-soluble fraction are water-soluble proteins. To determine if this was the case, disc electrophoretic patterns of the water-soluble protein, salt-soluble proteins and a mixture of both the salt and water-soluble protein are shown in figures 10 - 15. Examinations of the gel patterns showed that the patterns for the salt-soluble proteins contained a number of electrophoretically identical components as the patterns for the water-soluble protein. Apparently, the salt-soluble fraction contains some water-soluble protein due to incomplete separation during dialysis.

(iv) Alcohol-Soluble Proteins - Disc electrophoretic patterns of the alcohol-soluble proteins or "gliadins" of the extracted tetraploids, their parents, a durum wheat and mixtures of extracts from each extracted tetraploid and its parent are shown in Figures 16 - 20. The proteins of this solubility group migrated slowly into the gel giving mobilities in the R_f region from 0 to 0.3. A number of faint faster moving protein bands are also visible. These are probably water and/or salt-soluble proteins.

The pattern for the alcohol-soluble proteins of each extracted tetraploid was essentially the same as the pattern for its hexaploid parent. However, significant quantitative and qualitative differences between varieties were observed for this group of proteins. Thatcher, compared with the other two hexaploid wheats, had more protein bands. Differences were observed also between the durum wheat and the three bread wheat varieties. It is

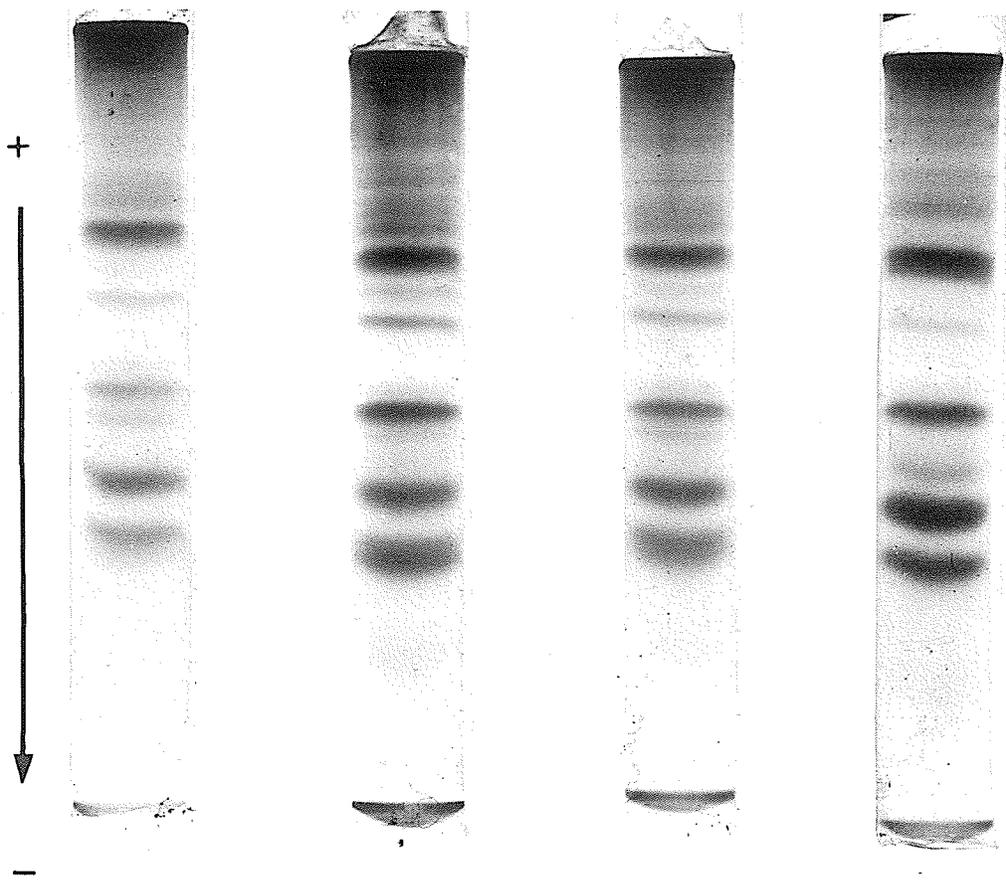
interesting to note that the pattern for the durum wheat (Stewart 63) was more similar to the patterns of Prelude and Rescue than Thatcher. Of the soluble proteins, the alcohol-soluble group appears to show the most marked quantitative and qualitative varietal and species differences. However, since the removal of the D genome did not affect the patterns of the three varieties studied, it appears that all the genes for this group of protein are located on the A and B genome chromosomes. Varietal differences in the patterns of this group suggest that marked evolutionary changes must have occurred in these genomes.

Because the alcohol-soluble group showed the most marked varietal differences, it was decided to examine them further by starch-gel electrophoresis. The patterns of the fractions from the extracted tetraploids and their parents are shown in Figure 21. As was found by disc electrophoresis, there were no significant differences in the patterns of each related pair of wheats. As with disc electrophoresis, significant quantitative and qualitative differences among varieties were observed. Thatcher showed three light leading protein bands while both Rescue and Prelude had only one, darkly stained, band in the same region.

A comparison of the starch-gel pattern (Figure 21) with the disc-gel patterns (Figures 16 - 20) demonstrates the superior resolving power of the disc electrophoresis technique. For example, the two slow moving, darkly stained protein bands of Thatcher in starch-gel electrophoresis appear as four distinct bands in disc-gel electrophoresis.

Since Prelude and its extracted tetraploid were similar in breadmaking quality, their alcohol-soluble proteins were studied in greater detail. Figures 16 - 18 show the disc electrophoretic patterns for this fraction from Tetraprelude, its parent and a mixture of equal parts of the two fractions examined at three different concentrations. Prelude showed two sharp protein bands in the R_f region from 0.10 to 0.12 with the first band being lighter in density than the second. Tetraprelude on the other hand had only one sharp protein band and possibly one faint band in this mobility region. In contrast to the results for Prelude, the first band of Tetraprelude was darker than the second. Even at higher protein concentration, it was not possible to demonstrate the existence of a distinct second protein band that was observed in extracts from Prelude. However, the mixture of the two gave only one dark protein band. It appears that the dark band of Tetraprelude and the dark band of Prelude fused together and ran as one. The resolution in starch-gel electrophoresis (Fig. 21) was insufficient to distinguish between these two proteins and they migrated as a single protein. It was not possible to demonstrate conclusively that Tetraprelude contained one less protein band than Prelude. However, a distinct difference in the staining intensity of the first two proteins (R_f 0.1 to 0.12) of Prelude and Tetraprelude was observed. Further work is required to improve resolution of these two proteins before a definite conclusion can be made on the difference of these two related species.

Fig. 22. Disc electrophoretic patterns for the glutenins from Tetraprelude (4xP), Prelude (6xP), Stewart 63 (ST63) and a mixture (M) of the glutenins from Tetraprelude and Prelude.



4xP

6xP

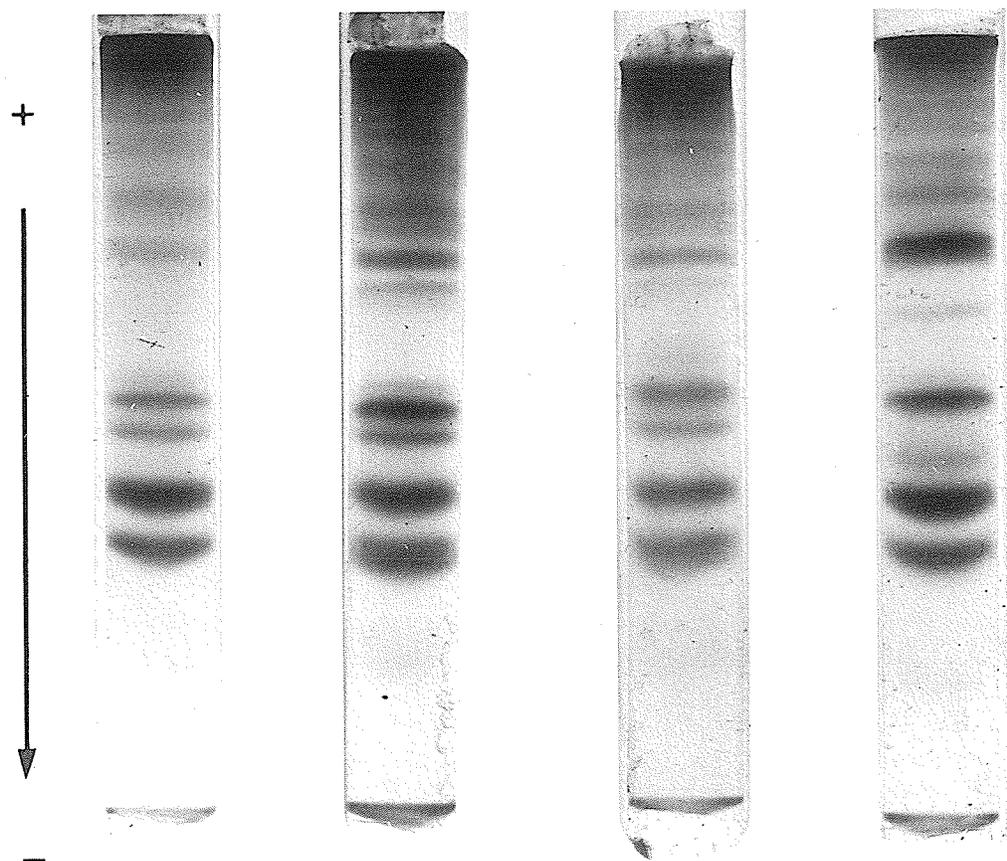
M

ST63

GLUTENINS

29

Fig. 23. Disc electrophoretic patterns for the glutenins from Tetrarescue (4xR), Rescue (6xR), Stewart 63 (ST63) and a mixture (M) of the glutenins from Tetrarescue and Rescue.



4xR

6xR

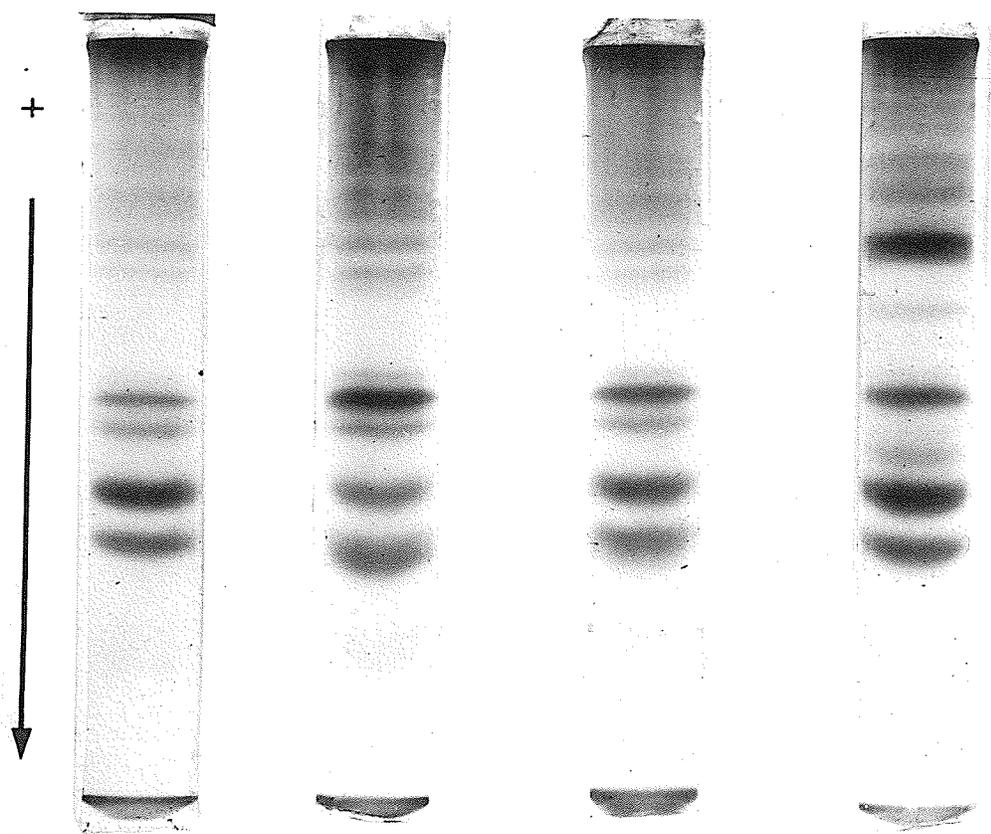
M

ST63

GLUTENINS

82

Fig. 24. Disc electrophoretic patterns for the glutenins from Tetrathatcher (4xT), Thatcher (6xT), Stewart 63 (ST63) and a mixture (M) of glutenins from Tetrathatcher and Thatcher.



4xT

6xT

M

ST63

GLUTENINS

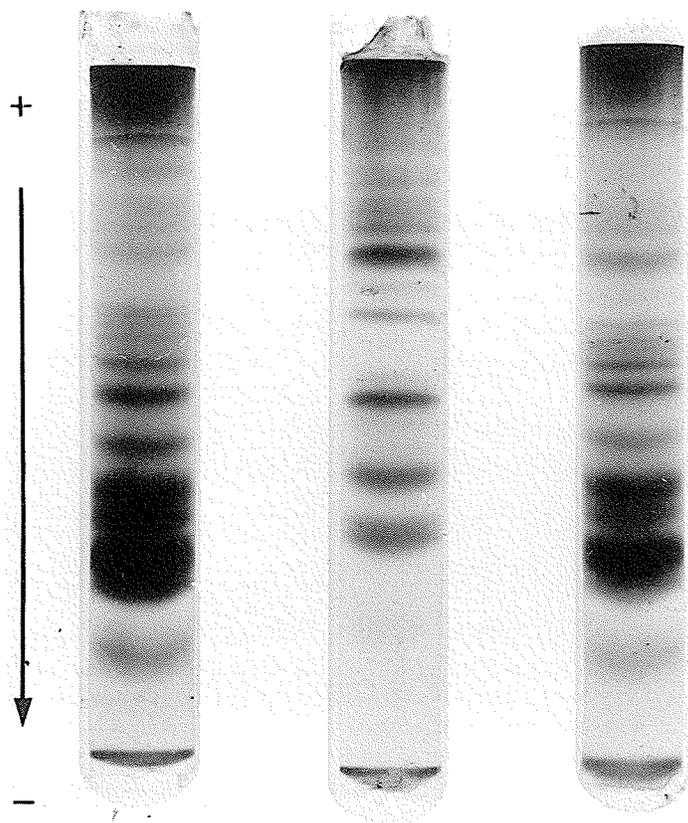
(v) Acetic Acid-Soluble Proteins - Figures 22 - 24 show the electrophoretic patterns of the acetic acid-soluble protein or "glutenins" of the extracted tetraploids, their parents, Stewart 63 and mixtures of the fractions from each extracted tetraploid and its parent. A much higher concentration (4 times that used with other fractions) had to be used to facilitate detection of the very light bands that resulted for this group of proteins.

The acid-soluble proteins are considered to comprise high molecular weight proteins which do not migrate into the gel. This was substantiated by the present study. In each case, a dark band was obtained at the origin. However, on examination of the gel patterns, four distinct fast moving protein bands were observed. These appear to be similar to the proteins in the water-soluble fraction. In addition the acid-soluble fraction appears to be contaminated with small amounts of alcohol-soluble proteins (R_f 0 to 0.3).

In order to ascertain if the fast moving proteins are the same as the water-soluble proteins, a mixture of the water-soluble and acetic acid-soluble fractions were examined for each extracted tetraploid and its hexaploid parent. The disc electrophoretic patterns are shown in Figures 25 - 30. In all cases, the four faster moving bands from the acetic acid-soluble fraction were identical with the proteins in the water-soluble fraction. Therefore, it can be concluded that the acetic acid-soluble protein fraction was contaminated with small amounts of water-soluble proteins.

(vi) Reduced Acetic Acid-Soluble Proteins - Disc electrophoresis experiments with the acetic acid-soluble proteins indicated that

Fig. 25. Disc electrophoretic patterns for the albumins (Alb),
the glutenins (Glu) and their mixture (M) from Prelude.



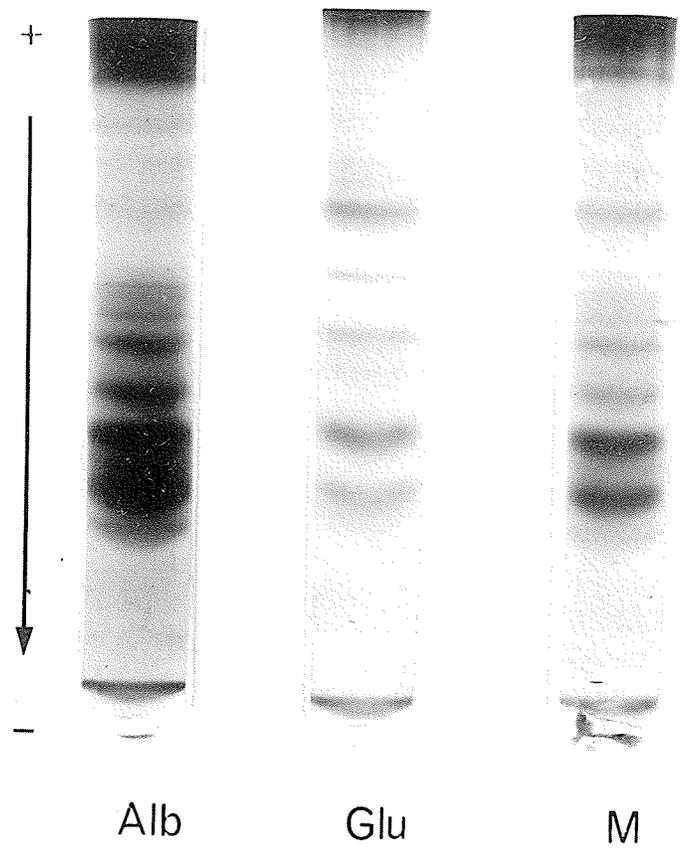
Alb

Glu

M

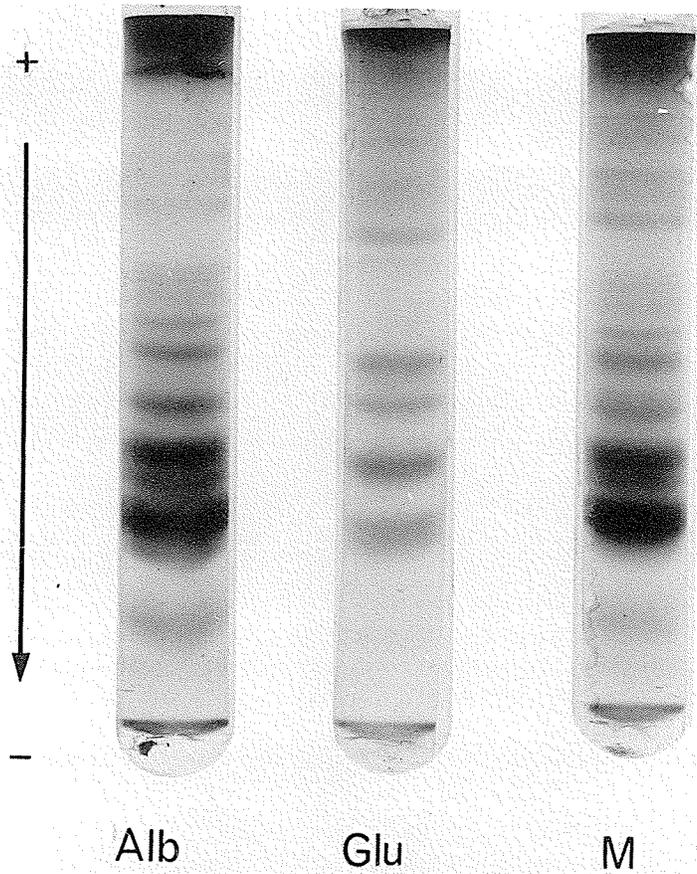
PRELUDE

Fig. 26. Disc electrophoretic patterns for the albumins (Alb), the glutenins (Glu) and their mixture (M) from Tetraprelude.



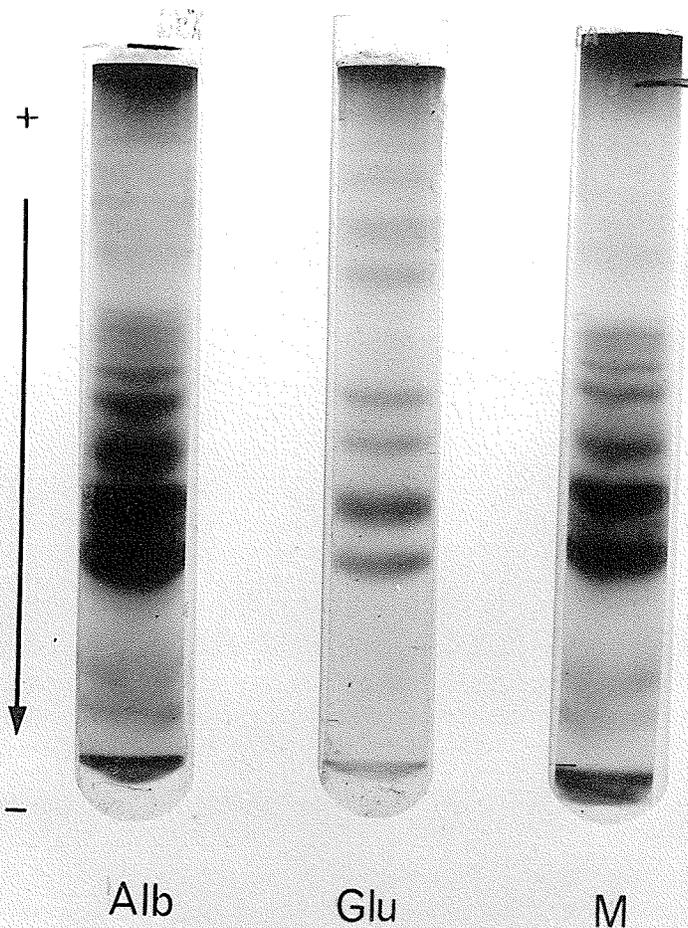
TETRAPRELUDE

Fig. 27. Disc electrophoretic patterns for the albumins (Alb), the glutenins (Glu) and their mixture (M) from Rescue.



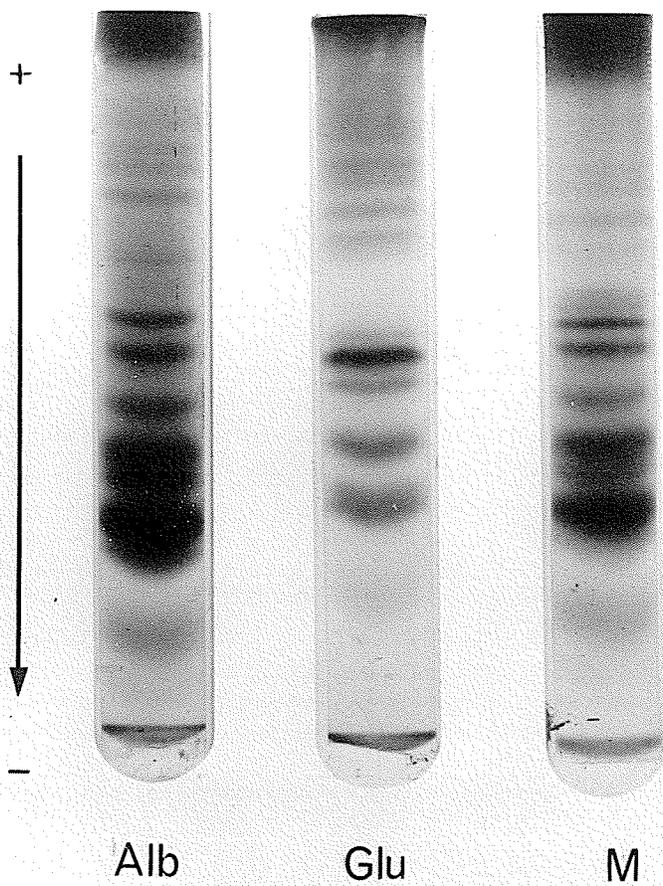
RESCUE

Fig. 28. Disc electrophoretic patterns for the albumins (Alb), the glutenins (Glu) and their mixture (M) from Tetra-rescue.



TETRA RESCUE

Fig. 29. Disc electrophoretic patterns for the albumins (Alb), the glutenins (Glu) and their mixture (M) from Thatcher.



THATCHER

Fig. 30. Disc electrophoretic patterns for the albumins (Alb), the glutenins (Glu) and their mixture (M) from Tetra-thatcher.

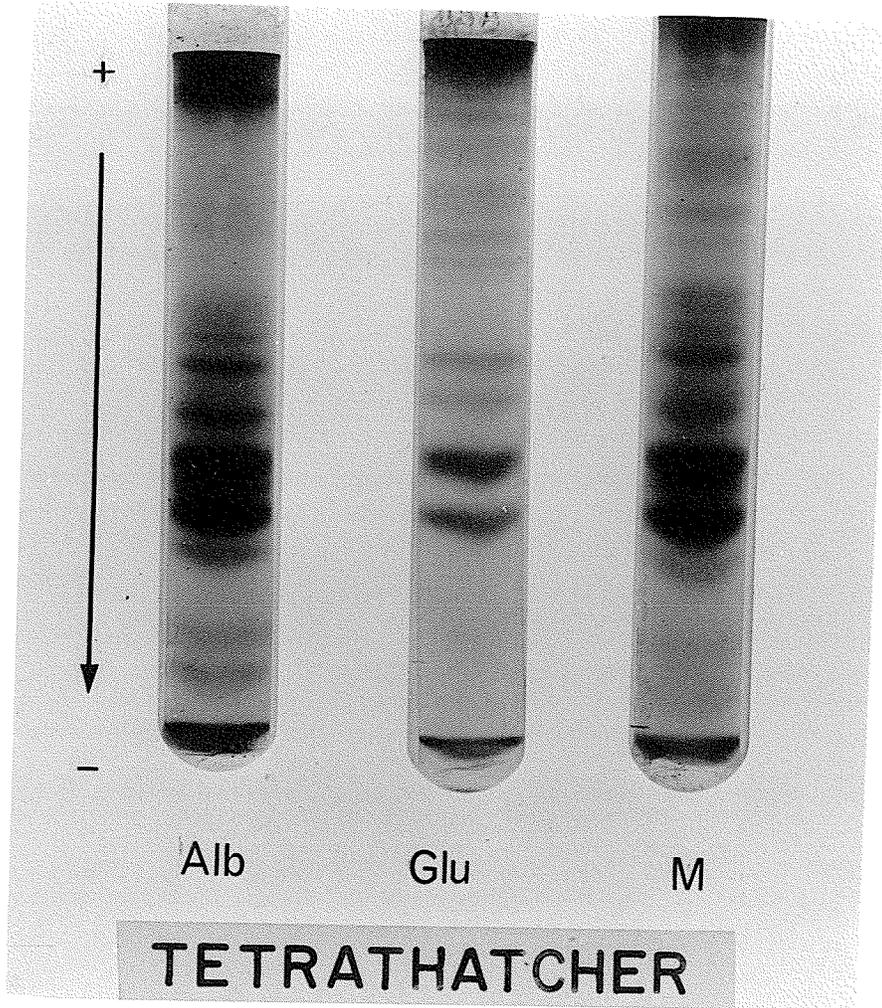
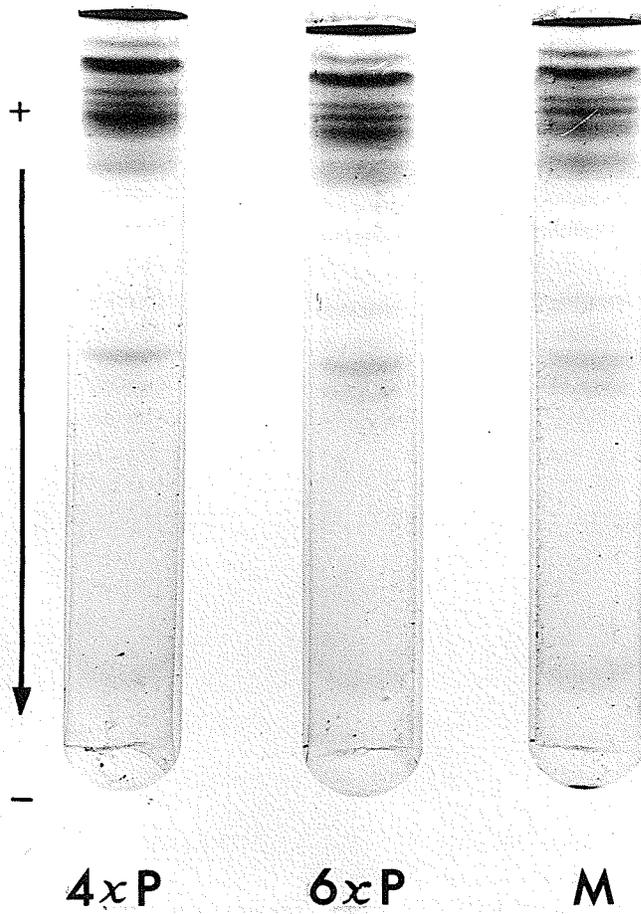
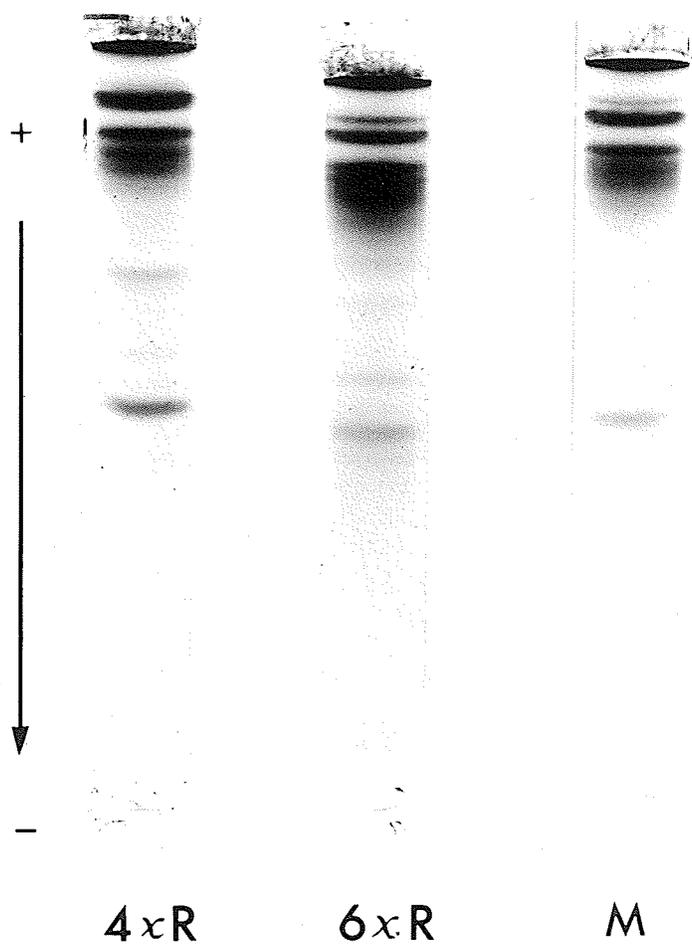


Fig. 31. Disc electrophoretic patterns for reduced-alkylated glutenins from Tetraprelude (4xP), Prelude (6xP) and their mixture (M).



REDUCED GLUTENINS

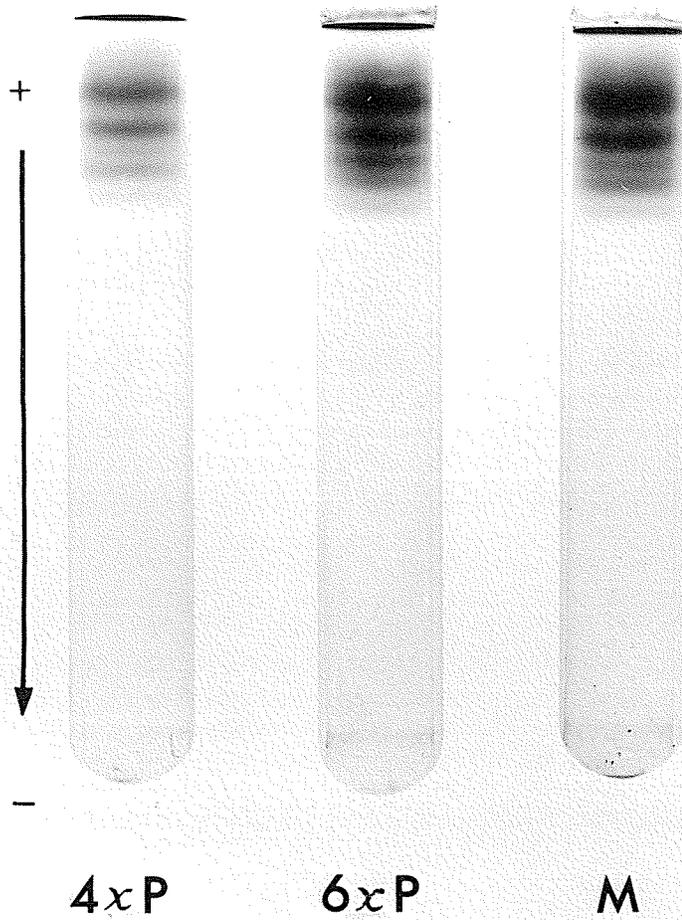
Fig. 32. Disc electrophoretic patterns for reduced-alkylated glutenins from Tetrarescue (4xR), Rescue (6xR) and their mixture (M).



REDUCED GLUTENINS

Fig. 33. Disc electrophoretic patterns for reduced-alkylated glutenins from Tetrathatcher (4xT), Thatcher (6xT) and their mixture (M).

Fig. 34. Disc electrophoretic patterns for reduced-alkylated gliadins from Tetraprelude (4xP), Prelude (6xP) and their mixture (M).



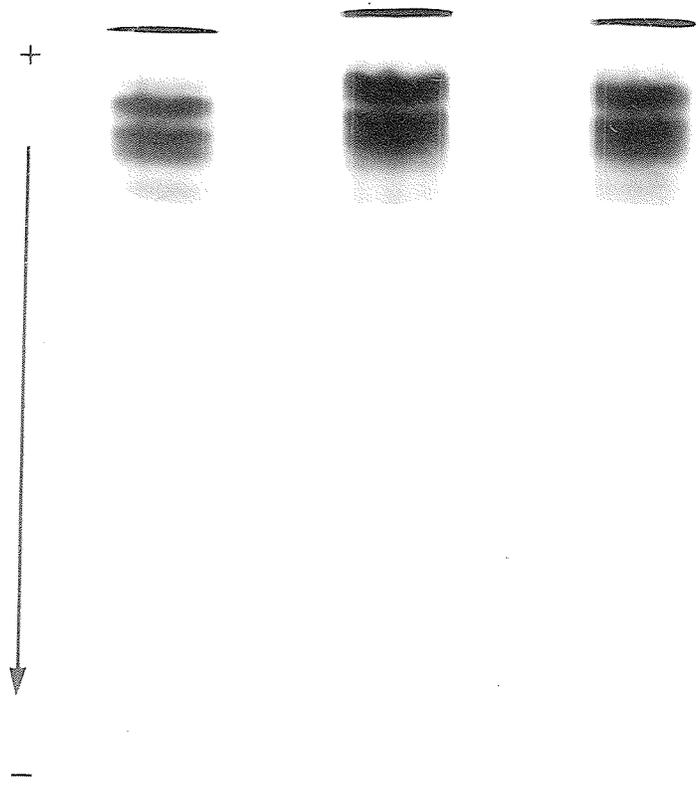
4xP

6xP

M

REDUCED GLIADINS

Fig. 35. Disc electrophoretic patterns for reduced-alkylated gliadins from Tetrarescue (4xR), Rescue (6xR) and their mixture (M).



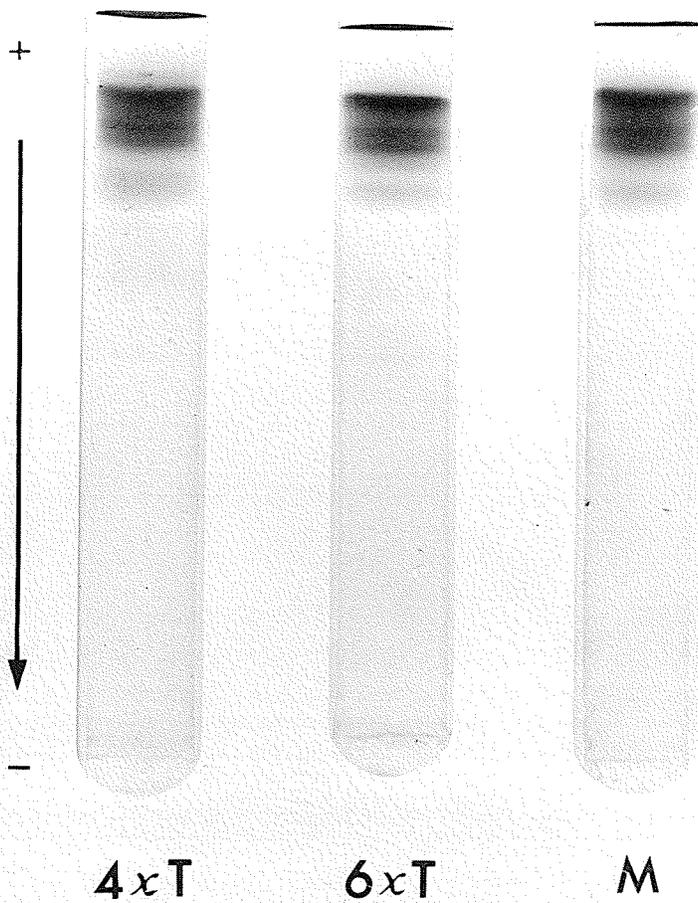
4xR

6xR

M

REDUCED GLIADINS

Fig. 36. Disc electrophoretic patterns for reduced-alkylated gliadins from Tetrathatcher (4xT), Thatcher (6xT) and their mixture (M).



REDUCED GLIADINS

most of the proteins of this fraction remained at the origin and could not be fractionated by this technique. Normally, this fraction comprises mostly glutenin proteins which are known to have high molecular weights (46). The large size of the molecules apparently prevented their penetration into the gel.

Reduction of the disulfide bonds of wheat glutenin sharply reduces its molecular weight to form a number of molecular species of similar size (41). These results indicate that smaller polypeptide chains are joined together by disulfide bonds to produce the large glutenin molecules (49). Fragments from reduced glutenin have been separated into a number of components by gel electrophoresis (42). Accordingly an attempt was made to apply this technique in conjunction with disc electrophoresis for a more detailed examination of the glutenin proteins of the extracted tetraploids and their parents.

Figures 31 - 33 show the patterns of the reduced and alkylated glutenins of the extracted tetraploid and their hexaploid counterparts. As found previously with starch-gel electrophoresis (42), the reduced and alkylated glutenin fragments migrated readily into the polyacrylamide gel. Most of the components were detected in the R_f region from 0 to 0.25. A dark band at the top of the gel indicated that some of the protein did not penetrate the gel.

Disc electrophoretic pattern of the reduced and alkylated glutenins of Tetraprelude was the same as that of its parent. However, the tetraploids of Rescue and Thatcher lacked one slow moving band (R_f 0.07) detected in the patterns for the parents. On the

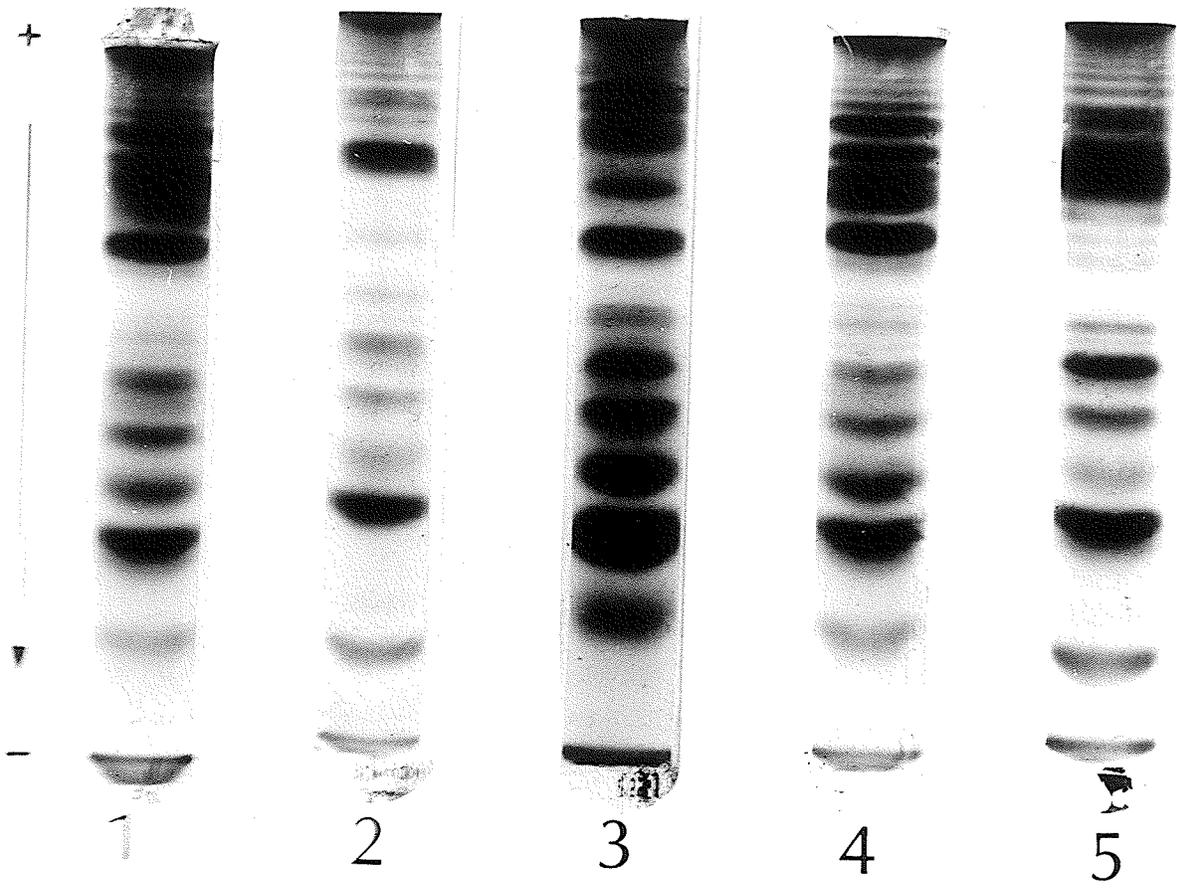
basis of these results, it might be concluded that the glutenins of Rescue and Thatcher are qualitatively different from the glutenins of the corresponding extracted tetraploids. This difference was not observed for Prelude and Tetraprelude.

In summary, it might be stated that the reduced and alkylated glutenins of the two extracted tetraploids with poor breadmaking quality lacked one slow moving band present in their hexaploid parents and in the extracted tetraploid of Prelude which had high breadmaking quality. It is tempting to conclude that this protein band might be partly responsible for breadmaking quality. However, since these differences were obtained for chemically modified glutenin which comprises only a small portion of the original glutenin protein this conclusion is tentative.

(vii) Reduced Alcohol-Soluble Proteins - Electrophoresis patterns of the reduced and alkylated alcohol-soluble protein (gliadins) of the three extracted tetraploids and their parents are shown in Figures 34 - 36. These proteins migrated very slowly into the gel and their mobilities were lower than those of the native gliadins. There were a number of identical bands in the patterns of the reduced and alkylated gliadin and native gliadin.

The patterns for the reduced and alkylated gliadins of the extracted tetraploids and their hexaploid parent were identical. Varietal differences in the patterns of the reduced and alkylated gliadins were observed as for native gliadin. On the other hand, the patterns of the reduced and alkylated gliadins were quite

Fig. 37. Disc electrophoretic patterns of the proteins from five varieties of Ae. squarrosa, 1. Strangulata 2. Anathera 3. Typica 4. Variety (1) 5. Variety (2)



different from those of reduced and alkylated glutenins Figures 31 - 33 for all the wheats studied. Accordingly, it appears that the reduced gliadins are different from reduced glutenin in electrophoretic properties. These results indicate that the gliadins are not the building blocks of the larger glutenin molecules as suggested by Woychik et al (42).

9. Electrophoretic Patterns of *Ae. squarrosa*

The breadmaking quality of hexaploid bread wheats has been attributed to the influence of the D genome chromosomes contributed by the primitive species *Ae. squarrosa* (82). Since the primary aim of the present study is to determine the contribution of the D genome to the protein composition of hexaploid wheats, it was desirable to examine the proteins of a number of representative species of *Ae. squarrosa*. It was presumed that gel electrophoresis patterns would provide information on the nature of proteins whose synthesis is controlled by the D genome in diploid cereal species.

Five accessions of the species *Ae. squarrosa* available in the Department of Plant Science, University of Manitoba were studied. Three of the accessions were of known identity and two were of unknown identity. Protein extracts from single seeds were examined by disc electrophoresis. Single seeds were ground, extracted with 4M dimethylformamide in 0.1N acetic acid and the insoluble-residue was separated by centrifugation. The proteins in the supernatant were examined by disc electrophoresis.

Disc electrophoresis patterns for extracts of the five accessions of *Ae. squarrosa* are shown in Figure 37. All five varieties

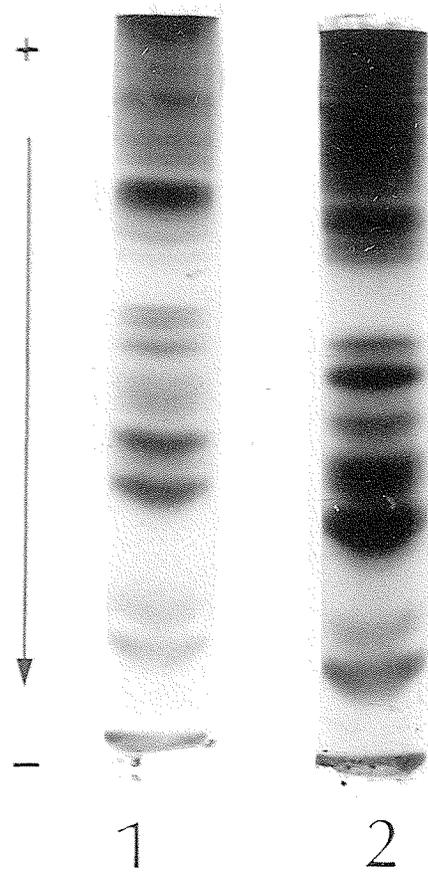
had a characteristic pattern. However, the albumin and globulin components gave identical patterns. These were similar to the patterns for the hexaploid and extracted tetraploid wheats discussed above. On the other hand, the gliadin portion of the pattern of all the Ae. squarrosa accessions differed markedly from the patterns of the analogous proteins in hexaploid wheat. These results agree, in general, with the findings of Boyd and Wrigley (69), that a wide diversity of protein patterns appears to exist in different species of Ae. squarrosa.

The findings of the present study indicates that the protein composition of Ae. squarrosa is similar to that of the hexaploid wheats examined. Accordingly, it appears that the D genome contributed by Ae. squarrosa is strongly expressed in hexaploid wheats. However, the effect of this genome must be primarily quantitative since it does not seem to have contributed any proteins that are qualitatively different from those present in AABB tetraploid. Furthermore, there is no evidence of the formation of hybrid proteins by the addition of the D genome to AABB tetraploids in the original formation of hexaploid bread wheat.

10. Disc Electrophoretic Patterns of the Proteins of a Synthetic AABBDD Hexaploid Wheat

To test the hypothesis that the contribution of the D genome to the proteins of bread wheat was quantitative rather than qualitative, the proteins of one line of synthetically produced hexaploid wheat (T. durum(Iumillo) x Ae. squarrosa) were examined by disc electrophoresis. Unfortunately the identity of the variety of

Fig. 38. Disc electrophoretic patterns of proteins from a synthetic AABBDD hexaploid and its durum parent.
1. Iumillo 2. Synthetic hexaploid.



Ae. squarrosa used in the initial cross is not certain, although it is presumed to be the variety Typica. Figure 38 shows that the electrophoresis patterns of extract from the durum wheat parent and the synthetic hexaploid are essentially identical. Apparently, no "new" proteins were contributed by the addition of the D genome from Ae. squarrosa. Recently, Boyd and Wrigley (69) examined two reconstituted hexaploids and found that the pattern for the reconstituted hexaploids were identical to the pattern for the mixtures of extract from the parents, and concluded that Ae. squarrosa contributed a number of proteins that were not present in the tetraploid. Further work is necessary to clarify this apparent discrepancy between the two sets of results.

11. Electrophoretic Patterns of Single Seed Protein Extracts

Recently, starch-gel and disc electrophoresis have been used extensively to separate and identify protein components in extracts from single grain seeds (68-70). Some of these reports concluded that control of the biosynthesis of certain proteins is associated with the 1D chromosome in the variety Chinese Spring (68). Moreover, on the basis of the same technique, Boyd and Wrigley (69) reported that extracted AABB tetraploids of Canthatch lacked a number of slow moving bands in the same region that was associated with chromosome 1D of Chinese Spring.

In present study of the extracted tetraploids and their parents, the proteins were extracted from flour in contrast to whole seed. Accordingly, it was felt that a comparison of the single whole seed

Fig. 39. Starch-gel electrophoretic patterns of 2M urea extracts of endosperm proteins of (1) the disomic parent and (2) nulli 1D-tetra 1A and (3) nulli 1D-tetra 1B aneuploid lines of Chinese Spring wheat.

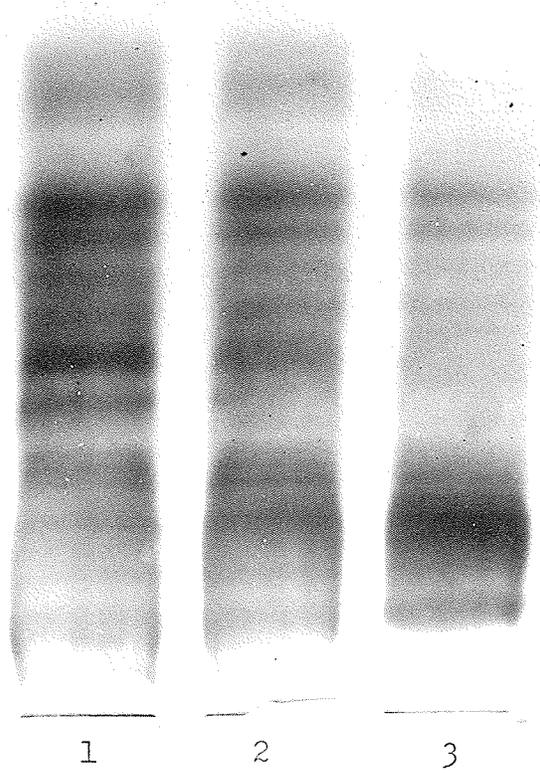


Fig. 40. Starch-gel electrophoretic patterns of 4M dimethylformamide - 0.1N acetic acid extracts of endosperm proteins of (1) disomic parent and (2) nulli 1D-tetra 1A and (3) nulli 1D-tetra 1B aneuploid lines of Chinese Spring wheat.

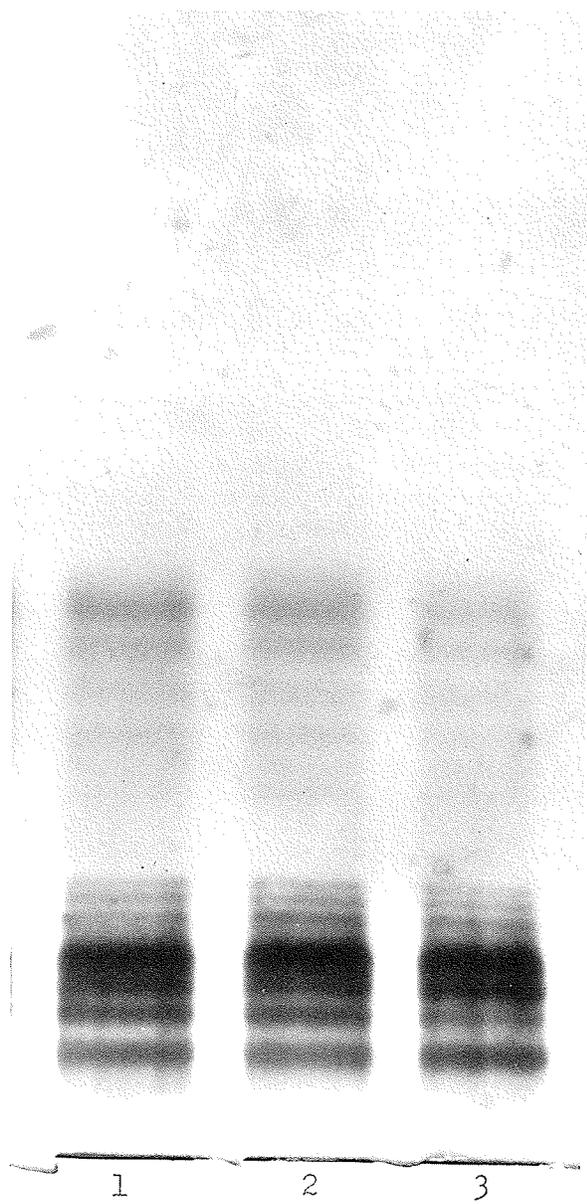
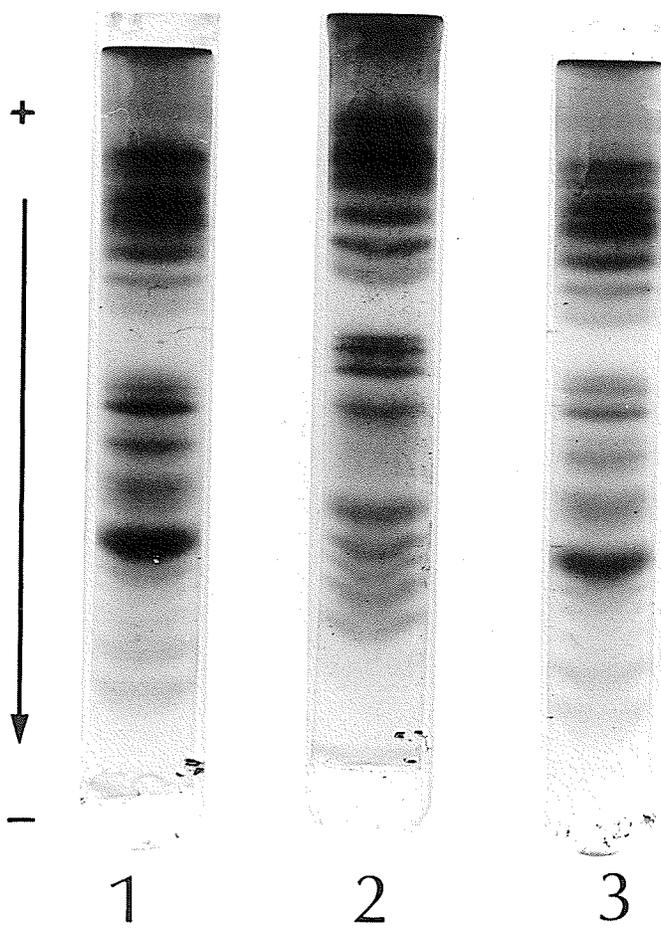


Fig. 41. Disc electrophoretic patterns of 4M dimethylformamide - 0.1N acetic acid extract of endosperm proteins of (1) disomic parent and (2) nulli 1D-tetra 1A and (3) nulli 1D-tetra 1B stocks of Chinese Spring wheat.



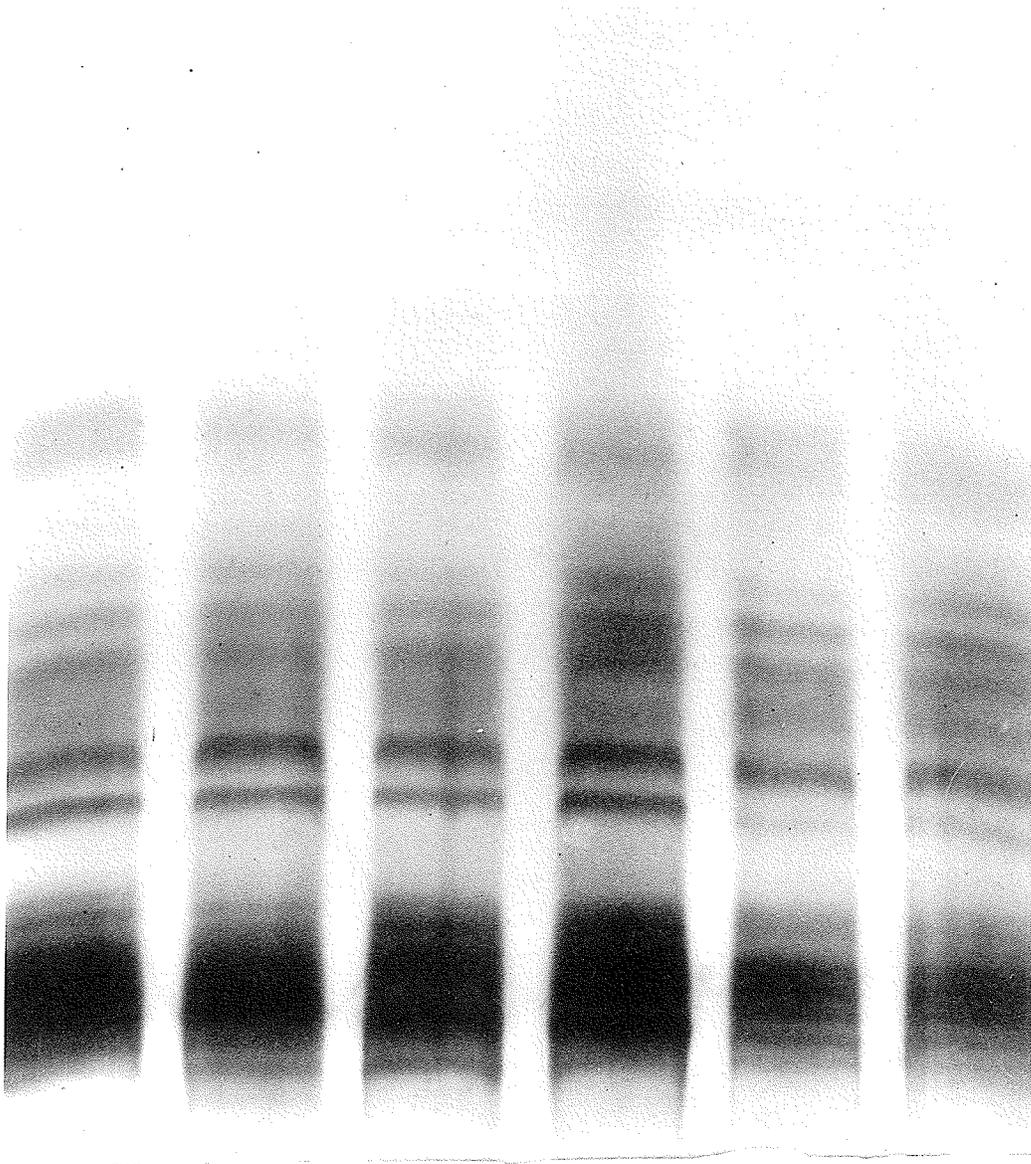
extracts and flour extracts for these wheats would be useful since there seems to be a discrepancy between the results of this study and those of Boyd and Wrigley (69). In addition two aneuploid lines and the disomic parent of Chinese Spring were examined to correlate the results of the two studies.

Two different solvents, 2M urea and 4M dimethylformamide in 0.1N acetic acid, were used to extract the proteins from single seeds. Figure 39 shows the starch gel patterns obtained for the urea extracts of two aneuploid lines of Chinese Spring and the disomic parent. It is apparent that the slow moving proteins are not separated completely. The reason for this is not known, however, there is a possibility that urea solubilized some of the lower molecular weight glutenins which would give rise to background staining as suggested by Elton and Ewart (43). The pattern for disomic Chinese Spring was identical to the patterns for the two compensating nulli-tetra aneuploids of the same variety. Due to lack of resolution in the slow moving bands it was impossible to ascertain if the two slow moving bands previously found by Shepherd (70) in the nulli 1D-tetra 1A and nulli 1D-tetra 1B aneuploids of this variety were absent.

Figure 40 shows the starch-gel patterns of extracts from the same two aneuploid lines and the disomic parent of Chinese Spring obtained with 4M dimethylformamide in 0.1N acetic acid. Again, the patterns for the three Chinese Spring lines were found to be identical. In this case, the slow moving proteins were completely separated and no background existed in the region of these bands

Fig. 42 Starch-gel electrophoretic patterns of 2M urea extracts of endosperm proteins of Tetraprelude, Tetrarescue, and Tetrathatcher and their hexaploid parents.

1. Tetrathatcher 2. Thatcher 3 Tetraprelude
4. Prelude 5. Tetrarescue 6. Rescue.



1

2

3

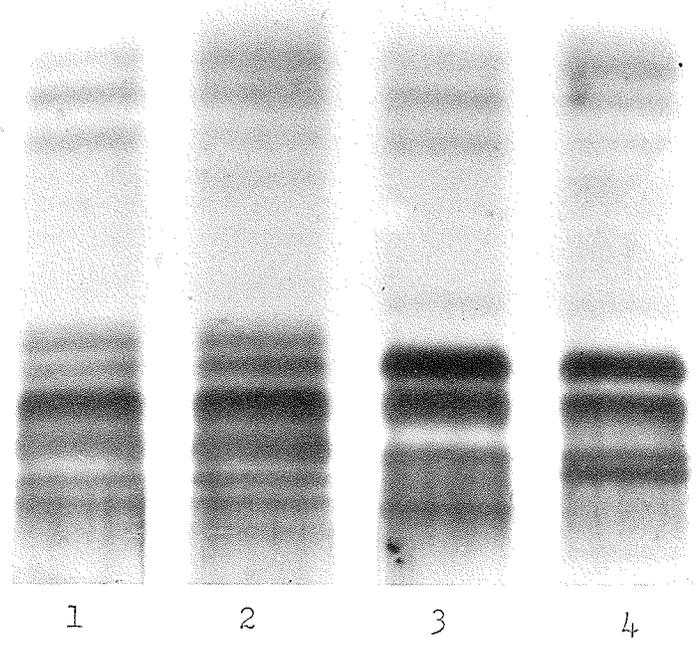
4

5

6

Fig. 43. Starch-gel electrophoretic patterns of a 4M dimethylformamide - 0.1N acetic acid extraction of endosperm protein of Tetrathatcher, Tetraprelude and their parents.

1. Tetrathatcher 2. Thatcher
3. Tetraprelude 4. Prelude



as was obtained with the urea extracts. The faster moving bands stained lighter than the same bands of the urea extracts.

Attempts to show the difference in the two slow moving proteins of Chinese Spring resulting from removal of chromosome 1D observed previously (70) were unsuccessful. The reason for this could be the low resolution of the protein components of low mobility when urea extracts are used.

As was found by starch-gel electrophoresis, the patterns for the dimethylformamide-acetic acid extracts of the two aneuploid lines of Chinese Spring and the disomic parent were also found to be identical by disc electrophoresis (Figure 41). Again, it was not possible to demonstrate the loss of two slow moving bands with removal of the 1D chromosome as reported by Shepherd (?). Further work is required to clarify this apparent discrepancy.

In addition to the single seed extracts of the two aneuploids of Chinese Spring, similar extracts of the three extracted AABB tetraploids and their parents used in this study were examined by starch-gel electrophoresis. The patterns of the 2M urea extracts were essentially the same for the extracted tetraploids and their hexaploid parents (Figure 42). As was found for the Chinese Spring aneuploids, the resolution in the slow moving region was not sufficient to produce a clear separation of bands.

More clear cut results were obtained with dimethylformamide-acetic acid extracts. Figure 43 shows the pattern of these extracts from the extracted tetraploid of Thatcher and Prelude and their hexaploid parents. The pattern of Thatcher and its extracted

tetraploid are identical, while the patterns for extracted tetraploid and hexaploid of Prelude showed a minor difference in the slow moving region. At the concentration of proteins used in this experiment, Prelude showed two intensely-stained bands in the region of low mobility while Tetraprelude had one intensely stained band and one light band of slightly lower mobility. Further work is necessary to ascertain if the observed differences for Prelude are real or an experiment artifact.

VI General Discussion

The proteins from three bread wheats and their extracted AABB tetraploids and a durum wheat were fractionated according to their solubility in water, salt solution, 70% aqueous ethanol and 0.05N acetic acid. The four soluble fractions obtained were identified as the albumins, globulins, gliadins and glutenins. This identity was based on solubility characteristics, amino acid composition, and electrophoresis mobility. Disc and starch-gel electrophoresis results showed that each group was a heterogeneous mixture of proteins that were characteristic of the solubility fraction and wheat variety. There was some overlapping, in the composition of the four groups, however, the amounts of contaminants as indicated by the gel electrophoresis patterns, were small.

The distribution of proteins among the four solubility groups for two of the three extracted tetraploids were significantly different from those of their hexaploid parents. Tetrarescue and Tetrathatcher contained more gliadin and glutenin, and less insoluble residue protein than the corresponding hexaploid varieties. This difference in solubility distribution of the proteins could be related to the inferior breadmaking quality of these two extracted tetraploids compared to their hexaploid parents. Accordingly, it is tentatively concluded that breadmaking quality of bread flours is determined by a critical balance of different proteins comprising the complex mixture present in the flours. This conclusion is supported by the results obtained for Tetraprelude and Prelude. With this variety, the extracted tetraploid was equal in breadmaking

quality to the hexaploid parent, and the solubility distributions of their proteins were essentially the same.

In addition to quantitative differences in the distribution of the proteins among solubility groups, there could also be qualitative differences between related wheats for a particular protein fraction. In the present study disc and starch-gel electrophoresis were used to establish identity of protein components. With these techniques, the proteins of each extracted tetraploid and its hexaploid counterpart were found to be quite similar. Thus it appears that the tetraploids have the necessary genes in the A- and B-genome chromosomes for the synthesis of all of the different species of protein found in their hexaploid parents. Apparently, the addition of the D genome to the tetraploid wheat did not add genes for "new" proteins. The main effect of this addition seems to be a decrease or increase in some of the proteins, present in the tetraploid wheats. In contrast, qualitative differences were observed between varieties of both tetraploids and hexaploid wheats.

It is interesting to note that the proteins of the Tetraprelude and Prelude were essentially the same as judged by the distribution among solubility groups and electrophoresis patterns of equivalent fractions. These results agree with breadmaking quality data. According to Kaltsikes et al (6), the retention of quality (and presumably quantitative composition of the proteins) was due to a translocation of a segment of chromosome 1D to one of the chromosomes of the A or B genome. It is perhaps somewhat surprising that a segment of one of the D chromosomes would be sufficient

to maintain the protein distribution necessary for breadmaking quality.

Protein components in the various solubility groups were characteristic for the varieties. Significant qualitative differences between varieties were observed in contrast to the similarity of the protein patterns of each extracted tetraploid and its hexaploid parent. It should be noted that these conclusions are based on results obtained with disc electrophoresis which resolves the proteins on the basis of their net charge per unit size. Accordingly, it is possible that relatively different proteins could have the proper combination of size and charge to have the same mobility. However, this is not very common. Unequivocal confirmation of identity of proteins of similar electrophoretic mobilities can only be made by a complete characterization of each protein in terms of amino acid sequence.

In the present study, only the proteins in the four solubility fractions were examined in detail. However, the insoluble residue contained about 30% of the flour proteins. The nature of these proteins remains to be resolved. It is possible that significant species and varietal differences might be found in this group of proteins. Elaborate degradation procedures would have to be used to study these highly insoluble proteins. The inability to separate the insoluble residue proteins may not be very serious in relation to breadmaking quality. In a recent study, Hosney et al (45) showed that the proteins that migrate in starch-gel are most important in breadmaking quality.

One other minor shortcoming of the present study that should be

mentioned is that each of the four solubility groups of proteins that were examined contained a small amount of protein that did not migrate into the gel. It is presumed that this arises from denaturation that normally occurs during freeze drying of solubilized proteins. It is presumed that the electrophoresis patterns are not affected qualitatively by this factor.

Of the soluble proteins, the alcohol-soluble group or gliadins showed the most marked qualitative and quantitative varietal and species differences. As indicated in the review of the literature, Hosney et al (45) found that the gliadin fraction plays an important role in determining the breadmaking potentialities of bread. This suggested that qualitative differences in gliadins might be important in baking quality of flour. So far it has not been possible to associate a particular electrophoresis pattern for the gliadin group with good breadmaking quality. Indeed, the present work which showed that the gliadin pattern of extracted tetraploids and original hexaploids were essentially the same, suggests that qualitative composition of the gliadins is not important in breadmaking quality. On the other hand, it was found that the poor quality tetraploids, like the normal durum wheats, contained much more gliadin fraction than the wheat of good quality. The extracted tetraploid of Prelude which had similar baking quality as hexaploid Prelude had essentially the same amount of gliadin as the parent.

In relation to inheritance of protein it is interesting to note that the patterns for the five accessions of Ae. squarrosa examined were quite different. This suggests that in the three varieties

examined, the variety of Ae. squarrosa that contributed the D genome contained protein components already present in the AABB tetraploids in the original formation of hexaploid wheats. It is also possible that the varietal differences among AABBDD hexaploid originated with varietal differences among the progenitors of the A and B genomes. If the D genome of each of the three varieties examined did contribute "new" proteins then the only explanation of the similarity in qualitative composition of the proteins of each extracted tetraploid and its parent would be on the basis of translocations of D genome chromosomes to those of the A and B genomes.

A translocation in one of the varieties studied (Prelude) involving a segment of the 1D chromosome was confirmed by Kaltsikes (75). No translocations were detected in the extracted tetraploids of Rescue and Thatcher. Recently, Mello-Sampayo (83) reported that spontaneous translocations occurred in a number of pentaploid hybrids between the chromosomes of the D genome and homoeologous chromosomes of the A and B genomes. If this occurred in the varieties studied, the proteins in the extracted tetraploid would be essentially identical. To rule out this possibility it is necessary to establish the absence of translocation in Tetrarescue and Tetrathatcher.

The results of the present study are at variance with those of Boyd and Wrigley (69). These workers found that removal of the D genome from Canthatch resulted in a loss of four protein bands. It is possible that the protein extraction procedures used in the two studies were sufficiently different to give the different results. Boyd and Wrigley (69) examined single seed extracts while in the

present work the flours were extracted consecutively by three different solvents. Perhaps the most plausible explanation of the discrepancy between the results of Boyd and Wrigley (69) and the results of this study is that different varieties were used in the two studies.

VII SUMMARY AND CONTRIBUTION TO KNOWLEDGE

1. Physicochemical properties of the proteins of AABB tetraploid wheats extracted genetically from three common wheat varieties Prelude, Rescue and Thatcher, their hexaploid parents and a durum wheat Stewart 63, were studied and compared.
2. In addition to the extracted tetraploids and their parents, protein extracts of two aneuploid lines and the disomic parent of the common wheat variety Chinese Spring, a synthetic AABBDD hexaploid wheat and its durum wheat parent, and five accessions of Ae. squarrosa were examined by gel electrophoresis.
3. The proteins of the extracted tetraploids and hexaploid counterparts were fractionated by solubility in appropriate solvents into albumins, globulins, gliadins, glutenins and insoluble residue proteins.
4. The distribution of the proteins among solubility groups showed that Tetrarescue and Tetrathatcher contained more gliadins, more glutenins, and less insoluble protein residue than the corresponding hexaploid wheats. In contrast, Tetraprelude contained similar amounts of gliadin, glutenin and insoluble protein residue to those in Prelude.
5. All four soluble fractions from extracted tetraploids contained more soluble material (protein and non protein) than the analogous fractions from the hexaploid parent varieties except the salt-soluble fraction was essentially the same for the seven wheats examined.

6. No significant differences in amino acid composition were found among the flours from the three extracted tetraploids, their hexaploid parents, and the durum wheat.
7. Amino acid composition varied significantly among the solubility fractions, however, the differences for any one fraction among the varieties and species were small.
8. Albumin and globulin proteins were rich in basic amino acids and poor in glutamic acid while gliadin proteins were characterized by high glutamic and proline contents and low lysine content.
9. Disc and starch-gel electrophoresis were used to separate and identify protein components of the various solubility groups.
10. The patterns obtained by the two techniques for each derived tetraploid were essentially the same as the equivalent patterns for the hexaploid counterparts. Two minor exceptions to this generalization were observed. Tetrarescue lacked one minor protein band in the albumin group, while both Tetrathatcher and Tetrarescue lacked one slow moving band in the reduced-alkylated glutenin group present in the analogous fraction of their hexaploid counterparts.
11. Differences in electrophoretic patterns were found among the varieties studied especially in the slower moving gliadin components, and in the reduced-alkylated glutenins.
12. Significant differences in the electrophoretic patterns were found in the gliadin proteins among the five accessions of Ae. squarrosa studied.

13. Similar electrophoretic patterns were obtained for the protein extracts from the synthetic AABBDD hexaploid wheat and the durum variety which contributed the A and B genomes.

VII REFERENCES

1. Morris, R. and Sears, E. R. The cytogenetics of wheat and its relatives. In Wheat and wheat improvement, K. S. Quisenberry and L. P. Reitz, eds., Monograph 13, Amer. Soc. Agron., Madison, Wisconsin (1967).
2. Pomeranz, Y. Relation between chemical composition and bread-making potentialities of wheat flour. 16:335-455 (1968).
3. Coulson, C. B., and Sim, A. K. Wheat proteins. I. Fractionation and varietal variation of endosperm proteins of T. vulgare. J. Sci. Food Agr. 16:458-464 (1965).
4. Doekes, G. J. Comparison of wheat varieties by starch-gel electrophoresis of their grain proteins. J. Sci. Food Agr. 19:169-176 (1968).
5. Lee, J. W., and Wrigley, C. W. The composition of gluten extracted from different wheats. Australian J. Exptl. Agr. Animal Husb. 3:85-88 (1963).
6. Kaltsikes, P. J., Evans, L. E., and Bushuk, W. Durum-type wheat with high breadmaking quality. Science 159:211-213 (1968).
7. Bailey, C. H. A translation of Beccaris' lecture "concerning grain" (1728). Cereal Chem. 18:555-561 (1941).
8. Osborne, T. B. The proteins of the wheat kernel. Carnegie Inst., Wash. Publ. No. 84 (1907).
9. Tiselius, A. A new apparatus for electrophoretic analysis of colloidal mixtures. Trans. Faraday Soc. 33:524-531 (1937).
10. Schwert, G. W., Putman, F. W., and Briggs, D. R. An electrophoretic study of gliadin. Arch. Biochem. 4:371-387 (1944).
11. König, P. Actas e Trabalhos do Tereiro Congresso Sul-Americano de Chimica, Rio de Janeiro et Sao Paulo 2:334 (1937).
12. Field, C. W., and Teagre, O. Electrical charge of the native proteins and the agglutinins. J. Explt. Med. 9:222-225 (1907).
13. Smithies, O. Zone electrophoresis in starch-gels: Group variations in the serum proteins of normal human adults. Biochem. J. 61:629-641 (1955).
14. Elton, G. A. H., and Ewart J. A. D. Starch-gel electrophoresis of wheat proteins. Nature 187:600-601 (1960).

15. Jones, R. W., Taylor, N. W., and Senti, F. R. Electrophoresis and fractionation of wheat gluten. *Arch. Biochem. Biophys.* 84:363-376 (1959).
16. Woychik, J. H., Boundy, J. A., and Dimler, R. J. Starch-gel electrophoresis of wheat gluten proteins with concentrated urea. *Arch. Biochem. Biophys.* 94:477-482 (1961).
17. Raymond, S., and Wang, Y. J. Preparation and properties of acrylamide gel for use in electrophoresis. *Analyt. Biochem.* 1:391-396 (1960).
18. Lee, J. W. Zone electrophoresis of wheat gluten on polyacrylamide gels. *Biochem. et Biophys. Acta* 69:159-160 (1962).
19. Bailey, J. L. *Techniques in protein chemistry.* Elsevier, New York (1967).
20. Wrigley, C. W. Analytical fractionation of plant and animal protein by gel electrofocusing. *J. Chrom.* 36:362-365 (1968).
21. Bailey, C. H. "The constituents of wheat and wheat products." Rheinhold, New York (1944).
22. Pence, J. W., Elder, A. H., and Mecham, D. K. Some effects of soluble flour components on baking behavior. *Cereal Chem.* 28:94-104 (1951).
23. Pence, J. W., and Elder, A. H. The albumin and globulin proteins of wheat. *Cereal Chem.* 30:275-287 (1953).
24. Pence, J. W., Weinstein, N. E., and Mecham, D. K. The albumin and globulin contents of wheat flour and their relationship to protein quality. *Cereal Chem.* 31:303-311 (1954).
25. Finney, K. F. Fractionating and reconstituting techniques as tools in flour research. *Cereal Chem.* 20:381-396 (1943).
26. Pence, J. W. The flour proteins. *Cereal Sci. Today* 7:178-180 (1962).
27. Hosney, R. C., Finney, K. F., Shogren, M. D., and Pomeranz, Y. Functional (breadmaking) and biochemical properties of wheat flour components. II. Role of water-solubles. *Cereal Chem.* 46:117-125 (1969).
28. Laws, W. D., and France, W. G. A comparative study of some protein fraction of wheat flour. *Cereal Chem.* 25:231-243 (1948).
29. Pence, J. W. Approximate isoelectric pH's of albumin and globulin proteins of wheat. *Cereal Chem.* 30:328-333 (1953).

30. Pence, J. W., Weinstein, N. E., and Mecham, D. K. Differences in the distribution of components in albumin preparations from durum and common wheat flours. *Cereal Chem.* 31:396-406 (1954).
31. Holme, J. Characterization studies on the soluble proteins and pentosans of cake flour. *Cereal Chem.* 39:132-144 (1962).
32. Elton, G. A. H., and Ewart, J. A. D. Starch-gel electrophoresis of cereal proteins. *J. Sci. Food Agri.* 13:62-72 (1962).
33. Elton, G. A. H., and Ewart, J. A. D. Electrophoresis comparison of cereal proteins. *J. Sci. Food Agri.* 15:119-126 (1964).
34. Nimmo, C. C., O'Sullivan, Mary T., Mohammad, A., and Pence, J. W. fractionation and zone electrophoresis of proteins of water-soluble materials of flour. *Cereal Chem.* 40:390-398 (1963).
35. Silano, V., de Cillis, V., and Pocchiari, F. Varietal differences in albumin and globulin fractions of T. aestivum and T. durum. *J. Sci. Food Agr.* 20:260-261 (1969).
36. Feillet, P., and Nimmo, C. C. Soluble proteins of wheat III. Isolation and characterization of two albumins (Alb. 13A and Alb. 13B) from flour. *Cereal Chem.* (in press).
37. Danielson, C. E. Seed globulins of the Gramineae and Leguminosae. *Biochem. J.* 44:387-400 (1949).
38. Fisher, N., Redman, D. G., and Elton, G. A. H. Fractionation and characterization of Purothionin. *Cereal Chem.* 45:48-57 (1968).
39. Abbott, D. C. What do we know about wheat proteins? *Cereal Sci. Today* 4:264-270 (1959).
40. Jones, R. W., Babcock, G. E., Taylor, N. W., and Dimler, R. J. Fractionation of wheat gluten by gel filtration. *Cereal Chem.* 40:409-414 (1963).
41. Nielson, H. C., Babcock, G. E., and Senti, F. R. Molecular weight studies on glutenin before and after disulfide bond splitting. *Arch. Biochem. Biophys.* 96:252-258 (1962).
42. Woychik, J. H., Huebner, F. R., and Dimler, R. J. Reduction and starch-gel electrophoresis studies of wheat gliadin and glutenin. *Arch. Biochem. Biophys.* 105:151-155 (1964).
43. Elton, J. A. H., and Ewart, J. A. D. Glutenins and gliadins: Electrophoretic studies. *J. Sci. Food Agr.* 17:34-38 (1966).
44. Huebner, F. R. Comparative studies on glutenins from different classes of wheat. 53th Ann. Meeting of AACC. Washington (1968).

45. Hosoney, R. C., Finney, K. F., Shogren, M. D., and Pomeranz, Y. Functional (breadmaking) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation *Cereal Chem.* 46:126-135 (1969).
46. Jones, R. W., Babcock, G. E., Taylor, N. W., and Senti, F. R. Molecular weight of wheat gluten fractions. *Arch. Biochem. Biophys.* 94:483-488 (1961).
47. Woychik, J. H., and Huebner, F. R. Characterization of gamma-gliadin *Fed. Proc.*, 47th ann. mtg. Fed. Amer. Soc. Exp. Biol., Atlantic City, N. J. April 16-20, 1963 Abstr. 22:477 (1963).
48. Pence, J. W., Nimmo, C. C., and Hepburn, F. N. Proteins In *Wheat: Chemistry and technology*, I. Hlynka, ed. American Association of Cereal Chemists, St. Paul, Minn. 227-276 (1964).
49. Wall, J. S. Review of wheat proteins. Fifth National Conference of Wheat Utilization Research, Fargo, North Dakota. 138-150 (1967).
50. Mills, G. L. Some observations on the electrophoresis of gliadin. *Biochem. Biophys. Acta.* 4:274-281 (1954).
51. Cluskey, J. E., Taylor, N. W., Charley, Helen, and Senti, F. R. Electrophoretic composition and intrinsic viscosity of glutens from different varieties of wheat. *Cereal Chem.* 38:325-335 (1961).
52. Graham, Janet, S. D. Starch-gel electrophoresis of wheat flour protein. *Aust. J. Biol. Sci.* 16:342-349 (1963).
53. Lee, J. W. and Ronalds, J. A. Effect of environment of wheat gliadin. *Nature* 214:844-846 (1967).
54. Huebner, F. R., and Rothfus, J. A. Gliadin proteins from different varieties of wheat. *Cereal Chem.* 45:242-253 (1968).
55. McDonald, C. E., and Gilles, K. A. Amino acid composition of wheat as related to quality. *Bakers' Dig.* 41:45-49 (1967).
56. Tkachuk, R. Amino acid composition of wheat flours. *Cereal Chem.* 43:207-223 (1966).
57. Sullivan, B. Wheat protein research-fifty years of progress. *Cereal Sci. Today* 10:338-344 (1965).
58. Lawrence, J. M., Day, Katherine M., Huey, Edith and Lee, Barbara. Lysine content of wheat varieties, species, and related genera. *Cereal Chem.* 35:169-178 (1958).
59. Woychik, J. H., Boundy, J. A. and Dimler, R. J. Amino acid composition of proteins in wheat gluten. *Agr. and Food Chem.* 9:307-310 (1961).

60. Ewart, J. A. D. Amino acid analysis of glutenins and gliadins. *J. Sci. Food Agr.* 18:111-116 (1967).
61. Johnson, B. L., and Hall, Ove. Analysis of phylogenetic affinities in the triticinae by protein electrophoresis. *Amer. Jour. Bot.* 52:506-513 (1965).
62. Hall, O., Johnson, B. L., and Olered, R. Evaluation of genome relationships in wheat from their protein homologies. *Proc. 2nd Int. Wheat Genetics Symp., Lund 1963, Hereditas, Suppl.* 2:47-54 (1966).
63. Johnson, B. L., Barnhart, D., and Hall, O. Analysis of genome and species relationships in the polyploid wheats by protein electrophoresis. *Amer. J. Bot.* 54:1089-1098 (1967).
64. Welsh, J. R., and Hehn, E. R. The effect of chromosome 1D on hexaploid wheat quality. *Crop Sci.* 4:320-323 (1964).
65. Morris, R., Schmidt, J. W., Mattern, P. J., and Johnson, V. A. Chromosome location of genes for flour quality in the wheat variety Cheyenne using substitution lines. *Crop. Sci.* 6:119-122 (1966).
66. Schmidt, J. E., Morris, R., Johnson, V. A., and Mattern, P. J. Comparison of the chromosome substitution and monosomic methods for wheat quality studies. *Crop Sci.* 6:370-372 (1966).
67. Welsh, J. R., Watson, C. A., and Green, C. W. Chromosomal control of flour properties in three substitution sets of common wheat. (Triticum aestivum L.) *Crop Sci.* 8:81-82 (1968).
68. Boyd, W. J. R., and Lee, J. W. The control of wheat gluten synthesis at the genome and chromosome levels. *Experientia* 23:332-333 (1967).
69. Boyd, W. J. R., and Wrigley, C. W. The D-genome and the control of wheat gluten synthesis. *Experientia* 25:317-319 (1969).
70. Shepherd, K. W. Chromosomal control of endosperm proteins in wheat and rye. *Proc. 3rd Int. Wheat Genet. Symp. Canberra, Aust.* 86-96 (1968).
71. Kerber, E. R., and Tipples, K. H. Effects of the D genome on milling and baking properties of wheat. *Can. J. Plant Sci.* 49:255-263 (1969).
72. Yong, F. C., and Unrau, A. M. Influence of alien genome combinations on protein synthesis in cereals. *Can. J. of Biochem.* 42:1647-1657 (1964).
73. Barber, H. N., Driscoll, C. J., Long, P. M., and Vickery, R. S. Protein genetics of wheat and homologous relationships of chromosomes. *Nature* 218:450-452 (1968).

74. Chen, C. H., and Bushuk, W. Nature of proteins in Triticale and its parental species III. A comparison of their electrophoretic patterns. *Can. J. Plant Sci.* (submitted).
75. Kaltsikes, P. J. Extraction and study of the AABB tetraploid component of three varieties of T. aestivum L. em. Thell and chromosome segregation in hybrids with the corresponding parental AABBDD hexaploid. PhD Thesis, University of Manitoba, 1968.
76. Kerber, E. R. Wheat: Reconstitution of the tetraploid component (AABB) of hexaploids. *Science* 143:253-255 (1964).
77. Jones, R. W., and Cluskey, J. E. Preparation of Aluminum-lactate. *Cereal Chem.* 40:589-591 (1963).
78. Kaminski, E., and Bushuk W. Wheat proteases. 1. Separation and detection by starch-gel electrophoresis. *Cereal Chem.* 46:317-324 (1969).
79. Davis, B. J. Disc electrophoresis II. Method and application to serum proteins. *Ann. N. Y. Acad. Sci.* 121:404-427 (1964).
80. Spackmann, D. H., Stein, W. H., and Moore, S. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* 30:1190-1206 (1958).
81. Yong, F. C. Influence of alien genome combinations in protein synthesis in cereals. Masters Thesis, University of Manitoba (1964).
82. Shebeski, L. H. Speculations on the impact of the D genome. *Proc. 1st Int. Wheat Genetic Symp., Winnipeg, Canada* 237-241 (1958).
83. Mello-Sampayo, T. Homoeologous chromosome pairing in pentaploid hybrid wheat, 3rd Int. Wheat Genetic Symp. Canberra, Australia. . 179-184 (1968).

