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A COMPARISON OF PROTEIN AND DNA IN DIFFERENT VARIETIES OF WHEAT (TRITICUM VULGARE)

by

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ABSTRACT

A comparison of protein and DNA of different hexaploid wheat varieties was carried out. Hard red spring wheats; Park, Thatcher and Neepawa, were selected, milled in a Buhler laboratory flour mill and flour fractions from the breaking and reduction rolls separately collected. From each flour, the proteins were successively extracted with water, 0.4 M sodium chloride in phosphate buffer pH 7.6 and with 70% ethanol to obtain the albumin, globulin and gliadin fractions. From separate flour samples, using 0.05 N acetic acid, soluble gluten proteins were extracted.

DNA extraction was achieved from green wheat leaves by a phenol extraction procedure. Contaminating proteins were removed by pronase digestion and chloroform-isoamyl alcohol treatment, while RNA was removed by pancreatic ribonuclease digestion.

Polyacrylamide disc gel electrophoresis in the presence of 3 M urea revealed for water, salt, and acid soluble proteins, some differences in the low and intermediate electrophoretic regions. Differences among gliadins were present only in the low electrophoretic mobility region. Flour from the breaking rolls contained more protein bands for most of the protein fractions than that from the reduction rolls.

Polyacrylamide disc gel elctrophoresis in the presence of sodium dodecyl sulfate revealed for wheat flour proteins, a molecular

weight range of from less than 17,800 to greater than 67,000. This range was represented in practically all protein fractions. Some differences were noted and were most striking between flours collected from the breaking and reduction rolls.

DNA isolated from all wheat varieties had a hyperchromicity value indicating native conformation. Melting temperatures in 0.015 M sodium chloride - 0.0015 M trisodium citrate, pH 7.0 were similar as were the calculated guanine plus cytosine values being 46.4 mole per cent for Park and Thatcher and 46.5 mole per cent for Neepawa. The thermal transition interval for each variety was 9.1°C. Using sodium chloride in concentrations of 0.005 - 0.5 M and potassium chloride of 0.008 - 0.5 M, a linear relationship between melting temperature and the negative logarithm of salt concentration used, was obtained. This relationship was independent of the wheat variety analyzed. The thermal transition interval width decreased as the salt concentration increased. Furthermore, DNA analysis using methylated albumin on kieselguhr did not reveal differences in DNA among the varieties.

A detailed comparison of the protein and DNA results obtained suggested that neither the protein fractions, nor the melting temperature determinations for DNA might serve as a reliable means of varietal differentiation within Titicum vulgare species.

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I. INTRODUCTION

Wheat, according to FAO data (1967), is the largest food crop in the world. Wheat flour possesses the unique ability of forming a dough that will expand by trapping and retaining gas produced by fermentation during bread production. One of the main concerns of the cereal chemist is that wheat flours from various varieties and species, vary in their ability to produce an acceptable loaf of bread.

The rheological properties of dough form the basis of farinographic and extensigraphic measurements for classifying a wheat variety as being suitable for breadmaking. With respect to their milling and baking characteristics, wheat varieties have been identified on the basis of their morphological and physiological characteristics, as well as on the basis of the milling properties of the kernels and on the rheological properties of the dough. However, as new wheat varieties are developed, these methods become less reliable due to the increasing overlap of properties possessed by these new varieties.

Much of the world wheat trade involves wheat which can be milled into flour possessing good breadmaking potential. Thus a country such as Canada can maintain her favorable position as a major supplier of the world's bread wheat only by ensuring that the buyer obtains the quality of wheat desired.

The ideal basis of varietal identification would be chemical or biochemical. Rheological properties of dough systems such as

extensigraphic, farinographic and baking tests have failed to provide a suitable and reliable varietal identification. Electrophoretic analysis of the proteins present in the endosperm also appears to be unreliable as a method of varietal identification. The experimental work to be described in this thesis is based on the idea of studying differences in the base composition of the deoxyribonucleic acid present in different wheat varieties, with the aim of developing a means of varietal identification.

II. REVIEW OF THE LITERATURE

A. THE GENEAOLOGY OF WHEAT

The ancestors of the modern bread wheats were diploids containing seven chromosomes. Triticum aegilopoides (wild einkorn) and Triticum monococcum (einkorn) contain the A genome (Mangelsdorf, 1953). Hybridization and chromosome doubling of these wheats with a seven chromosome wild grass, Aegilops speltoides, containing the B genome, resulted in the tetraploid wheats which contain fourteen chromosomes (Riley et al., 1958). An important tetraploid species is durum wheat which is used for the manufacture of paste products such as macaroni and spaghetti. An unusual tetraploid wheat is Triticum timopheevi, containing the G genome rather than a B genome. It is different from any of the other wheats containing fourteen chromosomes. An important characteristic of this wheat is the resistance to virtually all diseases attacking the other cultivated wheats.

Hexaploid wheats probably arose from the hybridization and chromosome doubling of a fourteen chromosome wheat species containing the A and B genomes with seven chromosome sets derived from the D genome, from Aegilops squarrosa (Riley, 1965). The hexaploid wheats Triticum aestivum (common), sphaerococcum (shot), and compactum (club), are the true bread wheats and account for over 90% of the wheat grown in the world (Mangelsdorf, 1953). Although these three species are closely related and easily intercrossed, it is not known whether they

were a product of three different hybridizations between fourteen chromosome wheats and wild grasses or from three diverging lines of descent from a single hybridization.

The geneaology of wheat has developed rapidly over the past decade as illustrated by the following figures. Figure 1 (Storck and Teague, 1952) illustrates one of the early presentations of the geneaology of wheat. Figures 2 (Mangelsdorf, 1953) and 3 (Riley, 1965) clearly indicate the progress made concerning the nomenclature and identification of the species which were parents of the modern hexaploid bread wheats.

There appears to be a chromosomal influence on the suitability of a wheat variety for breadmaking purposes. The D genome has been implicated in the inheritance of breadmaking quality. Boyd and Lee (1969), however, concluded that the D genome was responsible for four protein bands, three of low electrophoretic mobility and one of intermediate electrophoretic mobility. Dronzek et al. (1970) found that the presence of the D genome in hexaploid wheats does not result in the addition of new proteins to a wheat variety. The same authors found that tetraploid wheats and hexaploid wheats have the same flour protein patterns for the albumin, globulin, gliadin, and glutenin fractions. The different conclusions reached by Boyd and Lee and Dronzek et al. may be due to the extraction procedures used. Boyd and Lee used the procedure developed by Lee (1968) while Dronzek et al. used the consecutive extraction method used by Chen and Bushuk (1970). The two groups did not study the same varieties, which may also have an influence on the results.

Dronzek et al. (1970) concluded that the D genome appears to affect the amount of proteins originally present in the tetraploid

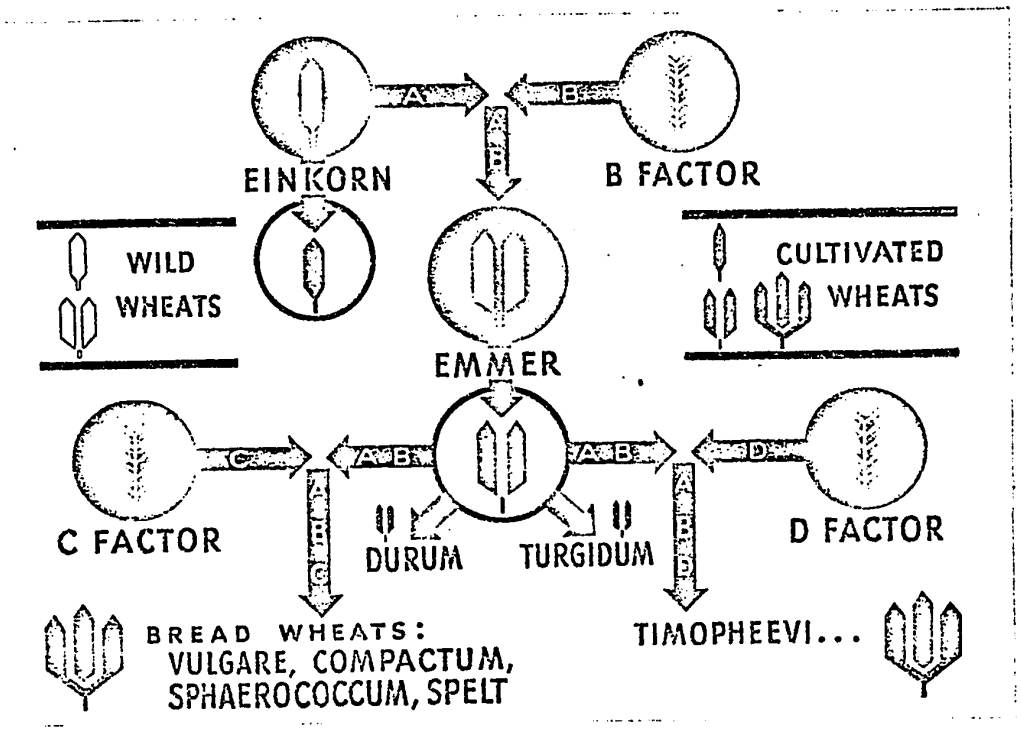


Figure 1

The Geneaology of Wheat (1952)

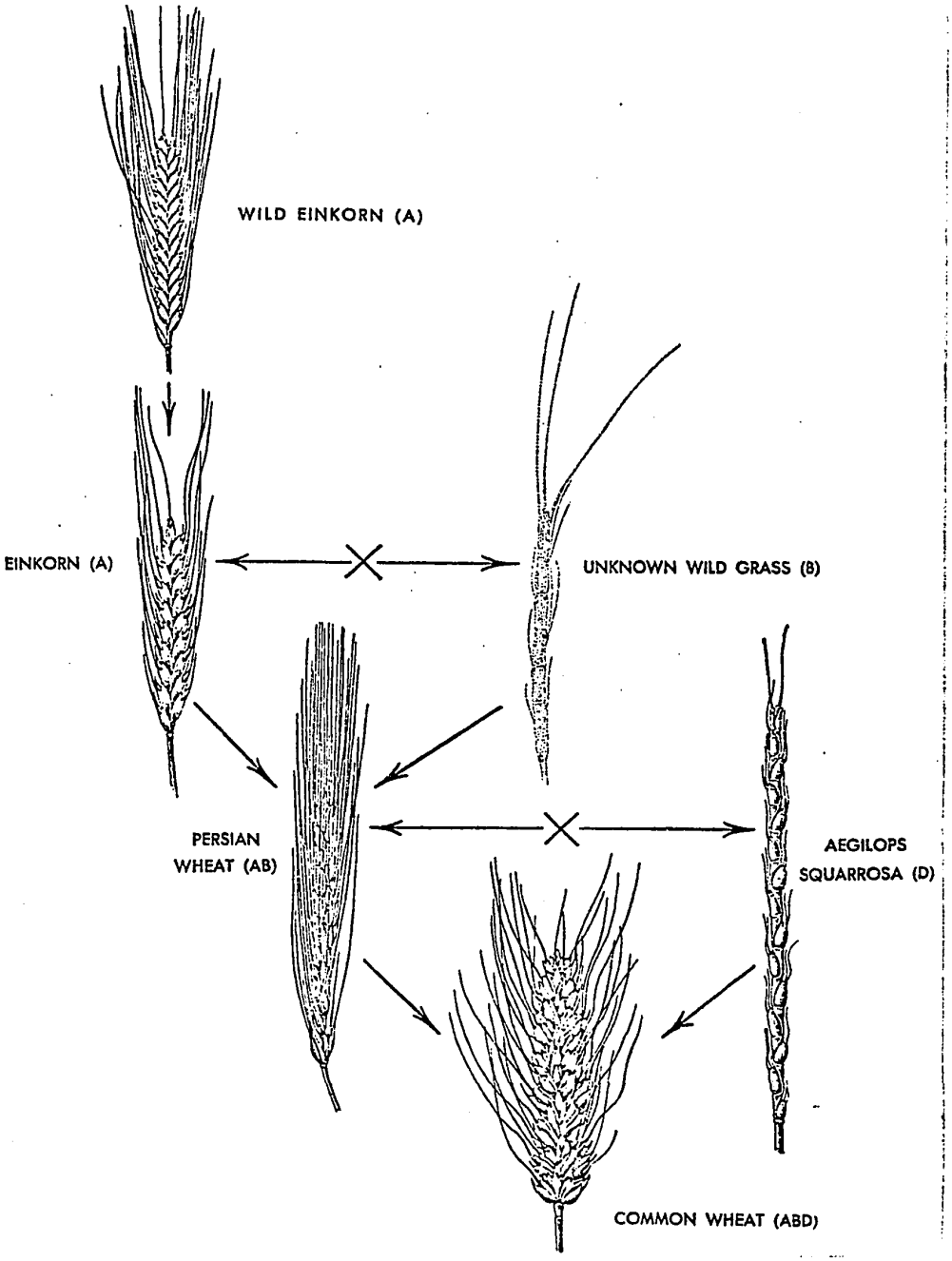


Figure 2

The Geneaology of Wheat (1953)

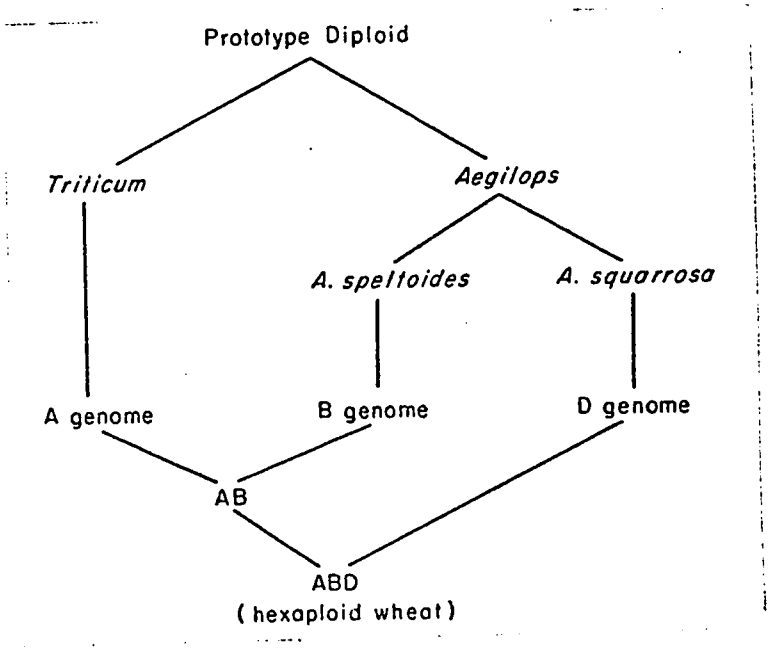


Figure 3

The Geneaology of Wheat (1965)

wheats and may result in the proper balance of different proteins of the flour, so necessary for good breadmaking qualities. However, Wrigley (1970) using a two-dimensional protein mapping technique of gel electrofocusing in one dimension and starch gel electrophoresis in the second dimension, found that the presence of the D genome in hexaploid wheats results in the presence of several gliadin bands not present in corresponding tetraploid varieties. This study used gliadins extracted with 2 M urea as did Boyd and Lee (1969), and appears to substantiate their results.

B. WHEAT PROTEINS

1. Extraction Techniques

Many of the studies involving wheat proteins are based on the solubility characteristics of the endosperm proteins. Osborne (1907) classified wheat proteins into four classes based on solubility properties. Albumins were soluble in water, globulins in salt solutions, gliadins in 70% ethanol (w/w), while the glutenins were insoluble in 70% ethanol but soluble in dilute acid or dilute alkali.

Many procedures exist for the extraction of proteins and protein classes from wheat flour and gluten. Pence and Elder (1953) described the purification of various protein classes through the use of fractional precipitation with ammonium sulfate. Gehrke et al. (1964) expanded and improved the methods of Pence et al. in order to characterize and study the homogeneity of the protein fractions of wheat flour. Ewart (1968) described a procedure for successive extraction of flour with 0.04 M sodium chloride, water, 70% ethanol, and 0.01 N acetic acid to obtain various protein fractions. A similar procedure

was used by Chen and Bushuk (1970) according to the following extraction sequence: two extractions with 0.5 M NaCl, one extraction using water followed by two extractions with 70% ethanol and two extractions with 0.01 N acetic acid rather than three extractions with each solvent as described by Ewart. Saline extraction solubilizes the albumins and globulins. Gliadins are slightly soluble in acqueous extracts with the high molecular weight gliadins being extracted in the 70% ethanol. Solubility of gliadins in aqueous extracts depends on the flour-water ratio used. Flour contains a considerable amount of salt, therefore the flour-water ratio determines the salt concentration and the amount and type of protein solubilized. Gliadins are least soluble when the flour-water ratio is high as they are relatively insoluble in dilute salt solutions. Flour-water ratios of 3:1 result in only traces of gliadins remaining in the acqueous fractions (Hoseney and Finney, 1971).

Urea extraction of gliadins after preliminary extraction of flour by water was described by Lee (1968). The gliadin extracted was reported to be relatively free of glutenin as demonstrated by gel filtration. This study indicated that the molecular weight range of the gliadin proteins is relatively narrow.

The need to have close control of conditions when extracting proteins from wheat flour was demonstrated by Jankiewicz and Pomeranz (1965) who studied the effect of the presence of urea and N-ethylmaleimide on the extractability of wheat flour protein. It was found that urea at pH 7.0 and 4°C solubilized almost all of the proteinacious material in wheat flour. The extent of hydrogen bonding and reactivity of free thiol and disulfide groups in the extraction system determined the

relative distribution and average molecular weight of the proteins extracted from the flour.

Meredith and Wren (1966) developed a solvent containing 3 M urea, 0.1 M acetic acid and 0.01 M cetyltrimethylammonium bromide (AUC) for the extraction of wheat proteins. This system was found to extract 95% of the total proteins of flour.

Maes (1962) developed a system for the extraction of wheat flour proteins on the basis of solubility. The procedure involved thoroughly mixing wheat flour and pumice (1:1). To this mixture they added ten times as much sand and the whole was mixed. The mixture was poured into a glass column containing a layer of quartz and a layer of sand. The mixture was covered with another layer of quartz and sand. About 60 ml of water was percolated through the column to wet it. Proteins were then eluted using 150 ml portions of distilled water, 10% isopropyl alcohol, 30% isopropyl alcohol, 4% lactic acid and 0.5% potassium hydroxide. This sequence of solvents separated the proteins into sharply defined fractions as the proteins were successively eluted by means of increasing concentrations of the same solvent in water or by a series of different solvents with an increasing ability to dissolve proteins. This procedure was modified somewhat by Mattern et al. (1968) who arrived at a new solvent sequence of 40% isopropyl alcohol, 2% NaCl, 3.85% lactic acid, and 0.1% potassium hydroxide which solubilized 94-97% of the protein in the flour. These authors found that 0.5% potassium hydroxide as used by Maes, resulted in an indistinct starch gel pattern which was interpreted as being due to protein denaturation. Extraction with 0.1% potassium hydroxide in the sequence of solvents,

resulted in a more distinct starch gel electrophoretic pattern.

2. Electrophoretic Studies

Electrophoretic studies of the proteins solubilized by various agents have been used extensively in attempts to correlate the protein composition of wheat varieties with rheological and varietal characteristics. The various types of electrophoretic techniques used were: electrophoresis according to Tiselius, starch gel and polyacrylamide gel electrophoresis as well as isoelectric focusing techniques.

Colvin and McCalla (1949) found that gluten dispersed in sodium salicylate solution gave rise to only one main peak and several very small peaks during electrophoresis according to Tiselius. Diffusion studies on gluten in sodium salicylate solution revealed that gluten dispersed in this solution had a mean surface charge density with a large, uniform, negative value, indicating that a gluten-salicylate complex was formed. Formation of such a complex may have raised the electronegativity of the gluten molecules to a point where small initial charge differences would be masked and unobservable during electrophoresis. Thus for an electrophoretic study of gluten proteins by means of Tiselius electrophoresis a solvent which did not complex with gluten would be required.

Jones et al. (1959) developed an aluminum lactate-lactic acid buffer for solubilization and electrophoretic separations of wheat proteins. They detected six gluten components applying Tiselius electrophoresis to gluten dispersed in various buffers including the aluminum lactate-lactic acid buffer, pH 3.1. These authors also studied electrophoretic patterns of gluten from Ponca, a hard red winter wheat,

and Lee, a hard red spring wheat; both having excellent baking qualities. Also studied were Willet, a hard red spring wheat, and Red Chief, a hard red winter wheat; both with poor baking qualities. The gluten from all four wheats produced the same electrophoretic patterns with all of the peaks in the same relative range of concentrations.

The aluminum lactate-lactic acid buffer was first applied to starch gel electrophoresis of gluten proteins by Elton and Ewart (1960). Electrophoresis of proteins soluble in 0.01 N acetic acid indicated the presence of eight distinct protein bands. It was concluded from this study that starch gel electrophoresis was a valuable method for the study of wheat proteins as eight well separated protein bands were obtained in comparison to the overlapping peaks obtained with Tiselius electrophoresis. The starch matrix also enabled separation on the basis of molecular shape and size as well as charge, rather than just on the basis of charge as in Tiselius electrophoresis.

Elton and Ewart (1962) began studies on the detection of differences in protein extracts of several wheat varieties. The fast moving electrophoretic components, the albumins and the globulins, showed similarities among varieties. This was in agreement with the results of Pence et al. (1954) who found strong resemblences in the electrophoretic patterns of wheat albumins after paper electrophoresis. Species differences were detected, but varietal differences were not observable. The question was raised as to whether the electrophoretic differences observed among species, were related to the inferior baking properties of the durum wheats. Jones et al. (1959) observed that the quantity and number of components differed among glutens from

different varieties of the same species.

Elton and Ewart (1960) were able to detect differences in the slow-moving gliadin fractions. The low mobility of these proteins during electrophoresis at acid pH was interpreted as an indication of high molecular weight and a low content of basic amino acid residues compared to the fast-moving albumins and globulins. The glutenins did not enter the starch gel as indicated by a dark patch at the origin after staining. The proteins used in this study were extracted by taking advantage of the solubility properties as described by Osborne (1907).

Coulson and Sim (1964), using starch gel electrophoresis and the aluminum lactate-lactic acid buffer system containing 0.5 M urea, examined the aqueous extracts of hand-ground samples of thirty-four varieties of Triticum vulgare. Major differences were found in the regions of low electrophoretic mobility. The overall protein pattern appeared to be typical of each variety. Differences due to location of growth were not evident. The protein patterns obtained could not be correlated with the rheological characteristics of the flours. Some species differences were observable.

Elton and Ewart (1964) examined the proteins of several wheat varieties extractable by water and by 70% ethanol, using starch gel electrophoresis. Qualitative and quantitative differences were evident in the gliadin and water soluble protein patterns among varieties. The electrophoretic patterns of oat, barley, rye and corn proteins were also examined. Rye was the only cereal with well marked bands in the mobility range corresponding to wheat glutens. Rye is the only other

cereal from which bread can be made. These results indirectly confirmed that the gluten proteins are responsible for the rheological properties of dough.

In the search for varietal differences, Coulson and Sim (1965) were able to separate the aqueous extracts of wheat flour into thirty-two components using starch gel electrophoresis. Several varieties of Triticum vulgare displayed distinct differences in the composition of fractions of low electrophoretic mobility. However, no correlation could be established between these differences and the rheological characteristics of dough made from the flour. As well, no obvious relationship was evident between closely related varieties. The suggestion was raised that these fractions may reflect the morphological characteristics of the variety and may be influenced to a small extent by environmental factors. It was concluded that fractions of intermediate electrophoretic mobility were probably distinctive to both species and variety.

Doekes (1968) examined the starch gel electropherograms of the aqueous extracts of eighty different wheat varieties. On this basis the varieties could be divided into five main groups which formed a morphological series. Each group was composed of a number of sub-groups, some of which were based on genetic relationships. The gliadin patterns were used to place a variety in a main group after which it was found that classification into sub-groups was possible in some cases on the basis of differences in the patterns of the components of high and intermediate electrophoretic mobility.

Huebner and Rothfus (1968) examined the protein composition

of the gliadin fractions from several varieties of common, durum,and club wheats by column chromatography on sulfoethyl cellulose and by starch gel electrophoresis. Findings revealed that compositional differences were greatest between varieties of different classes and smallest between varieties of the same class. Amino acid analyses of the gamma-gliadins from different varieties were almost identical.

Huebner (1970) studied the glutenins of eleven varieties of wheat representing five different classes. Glutenins were isolated by gel filtration on Sephadex G-100 from a 0.05 N acetic acid extract of defatted gluten. Starch gel electrophoresis, with a 3 M urea, aluminum lactate buffer at pH 3.1; of reduced, alkylated glutenins, showed some variations among varieties of the same class. The greatest variations, however, were among classes.

The Maes' procedure was used by Williams and Butler (1970) to extract proteins from flour from several varieties using the solvent sequence described by Mattern et al. (1968). Protein patterns of the various fractions were analyzed using polyacrylamide disc gel electrophoresis at pH 4.5 and at pH 8.9. Cationic electrophoresis at pH 4.5 resulted in sharper resolution of protein components than anionic electrophoresis at pH 8.9. However, the higher pH revealed more clear-cut differences between flour samples.

Electrophoresis at pH 8.9 revealed species differences but varieties of the same species showed very little difference in electrophoretic patterns. Clear-cut differences were observed between tetraploid and hexaploid wheat types. Within species of the same chromosome number, the electrophoretic distribution of proteins was very similar.

Hexaploid wheat varieties of widely varying rheological properties had proteins remarkably alike in electrophoretic properties. Little difference was apparent in the number of components or their mobility among proteins from soft white winter, soft white spring, hard red spring, and hard red winter wheats.

As a result of these studies, Williams and Butler (1970) held the view that the electrophoretic patterns are more likely to be associated with the genetic constitution of the variety and its' parents, than with the rheological and baking properties of the flours obtained from these wheats. Some evidence was presented indicating that proteins extractable by 3.8% lactic acid used in the sequence of solvents of the modified Maes' extraction procedure, may yield more specific differences among wheat types than the other solvent system. The lactic acid-soluble proteins of Thatcher differed from those of other hard red spring types, although the authors were unable to relate these differences to any particular aspect of flour quality. Although Manitou and Thatcher had similar baking qualities, they differed in electrophoretic behavior. Manitou was identical to Selkirk and Garnet in electrophoretic behavior, although the latter two varieties differed from Manitou and Thatcher in flour quality.

Wrigley (1968) applied the technique of gel electrofocusing to wheat gliadins. Heterogeneity of the gliadin proteins separated by this technique was similar to that found previously by gel electrophoresis. The techniques of starch gel electrophoresis and gel electrofocusing were combined by the same author, into a two-dimensional technique for the study of varietal differences in the gliadin fraction

of wheat proteins. The gliadins were extracted from a wheat kernel using 0.4 ml of 2 M urea. The clarified extract was separated in the first dimension by gel electrophocusing between pH 5 and pH 9. After electrofocusing, the unfixed electrofocused gel was inverted into a slot across one end of a starch gel for electrophoresis according to Graham (1963). Over forty components were detected by using this technique. In the two-dimensional map obtained, horizontal displacement of the proteins gave an indication of the isoelectric point while vertical displacement in the starch gel varied inversely with molecular size and directly with the charge used in electrophoresis (pH 3.1). This technique revealed considerable differences in the gliadin composition of different varieties of bread wheats, as many single zones obtained by either technique alone, were shown to consist of several components after application of the two-dimensional technique.

3. Electrophoresis in the Presence of Sodium Dodecyl Sulfate

One of the problems associated with electrophoresis of proteins is that electrophoretic mobility is determined by a combination of molecular size and charge. Thus it is conceivable that two proteins differing in molecular size and charge at the pH of electrophoresis could have the same electrophoretic mobility. Shapiro et al. (1967) studied the possibilities of electrophoretically determining the molecular weight of proteins treated with the anionic detergent, sodium dodecyl sulfate (SDS). Treatment of a protein with SDS and 2-mercaptoethanol results in extensive disruption of hydrogen, hydrophobic, and disulfide linkages. Complex formation between the protein and SDS minimizes the native charge differences. As a result, the protein

then behaves as an anion and migrates at a rate determined almost solely by the molecular size. It was found that in 5% polyacrylamide gels, proteins of known molecular weight ranging from 15,000 to 90,000 displayed a linear relationship between the rate of migration and the molecular weight.

Weber and Osborn (1969) concluded that the SDS polyacrylamide electrophoresis was a reliable method for the molecular weight determination of a wide variety of proteins when the logarithm of the molecular weight was plotted as a function of electrophoretic mobility. An interesting aspect of this is that electrophoresis of wheat proteins under these conditions may reveal varietal differences that may be masked due to the influence of charge and molecular size of the proteins under normal electrophoretic conditions.

4. Gel Filtration and Column Chromatography

Proteins from wheat flour have also been analyzed by column chromatographic methods. Simmonds (1963) used DEAE cellulose to separate proteins soluble in 0.01 M sodium phosphate buffer, pH 7.0. Eight subfractions were obtained and a comparison of the elution patterns of ten different flours revealed a marked difference in the relative amounts of the various components present. Protein fractions have also been obtained from gluten by means of gel filtration with Sephadex as described by Jones et al. (1963) who obtained six fractions differing in average molecular weight. None of the fractions were homogeneous when examined by starch gel electrophoresis.

Meredith and Wren (1966), using the AUC solvent developed by them, obtained four peaks from gluten solubilized in AUC by gel

filtration using Sephadex G-200. The peaks obtained were the glutenin, gliadin, and albumin peaks, and a non-protein peak.

Wright et al. (1964) used Sephadex G-100 to separate the aluminum lactate extracts of flour and gluten into about four protein groups of different molecular weight ranges.

Booth and Ewart (1969) fractionated wheat gliadin into four chromatographically pure components through the use of fractional precipitation of gliadins by ammonium sulfate to remove albumin and globulin impurities. Column chromatography of pure gliadin on carboxymethyl cellulose using a convex sodium chloride gradient resulted in four fractions being obtained. Ultracentrifugation of the proteins indicated molecular weights in the range of 75,000 to 79,000. Fingerprinting of tryptic and chymotryptic digests indicated that all four proteins had a similar primary structure. Small varietal differences due to a variation in amino acid sequence may have been present. The high glutamine and proline contents of these proteins suggested that the gliadin proteins may be storage proteins for nitrogen for the germinating embryo.

5. Effects of Nitrogen Fertilization

Doekes (1968) examined the effects of nitrogen fertilization on the starch gel electrophoretic patterns of wheat proteins. Increases in the protein content of flour due to nitrogen fertilization had no observable effect on the amount of albumins and globulins, whereas the level of gliadins increased. The electrophoretic pattern was unaffected.

Lee and Ronalds (1967) found that the combined effects of

differences in locality, soil type, and environment, resulting in as much as two-fold increases in total protein content of the flour, had little effect on the distribution of the gliadin components. thus varietal differences appear to be much more important than environmental factors on the starch gel electrophoretic patterns of wheat flour proteins. It is possible that the electropherogram of the proteins of a wheat variety constitutes a genetic image of the wheat, which is in agreement with the conclusions of Williams and Butler (1970).

C. NUCLEIC ACIDS

The current thought is that the protein patterns observed upon electrophoretic separation of wheat proteins is an image of the genetic makeup of the wheat variety (Lee and Ronalds, 1967; Williams and Butler, 1970; Doekes, 1968; Wrigley, 1970). However, since many of the major proteins of the wheat endosperm have no known biological function, they are considered storage proteins. Thus mutational changes may have resulted in a great number of similar proteins, as the possibility of mutational changes in storage proteins would be greater than with enzymes, due to fewer physical constraints (Booth and Ewart, 1969). These mutational changes may have resulted in the marked similarity of proteins between wheat varieties.

All of the genetic information of any living organism is present in the deoxyribonucleic acid (DNA). Any varietal differences would be coded in the DNA and a study of wheat DNA would appear to be a useful tool towards wheat varietal identification. However, very little work has been carried out on the DNA of wheat varieties with the

aim of varietal identification, although many well established physical and chemical methods have been developed for the analysis of DNA and nucleic acids in general.

1. Isolation and Purification

Many methods for extraction and isolation of DNA are available, the most accepted probably being that of Marmur (1961), developed for use with microorganisms. This procedure was used for the extraction of DNA from wheat embryos by Chen et al. (1968). Hotta and Bassel (1965) described the isolation of nuclei from wheat embryos and the subsequent extraction of DNA from these nuclei. After sedimentation analysis and electron microscopy, they concluded that this DNA was of high molecular weight.

The extraction of nucleic acids from freshly harvested wheat leaves was described by Hadziyev et al. (1969). The isolation of nuclei from the leaves of germinated wheat was described by Huguet and Jouanin (1972) who subsequently isolated the DNA from the nuclei following the method of Marmur (1961).

Most methods of DNA isolation are based on disruption of cells and nuclei by anionic detergents, phenol, or by mechanical means. Removal of proteins is accomplished by phenol denaturation, protein precipitation by chloroform-isoamyl alcohol (Sevag et al., 1938), or enzymatically by pronase digestion (Hotta and Bassel, 1965). Removal of ribonucleic acid (RNA) from the DNA preparation can be accomplished by means of preferential solubilization of RNA in isopropanol (Marmur, 1961), or by ribonuclease digestion (McCarty and Avery, 1946).

2. Methylated Albumin on Kieselguhr Column Chromatography

Mandel and Hershey (1960) developed methylated albumin on kieselguhr column chromatography for the fractionation of nucleic acids. The column fractionated DNA on the basis of molecular size, hydrogen bond content and base composition. It was also capable of separating DNA from RNA and transfer RNA from ribosomal RNA. Separation of nucleic acids was achieved with a linear gradient of increasing sodium chloride concentration. The nucleic acids were bound to the column material by salt linkages from neutral solutions that were 0.4 molar or less in sodium chloride. Each nucleic acid species was then eluted at a salt concentration characteristic of that species.

In addition to the continuous gradient, stepwise elution with various salt concentrations was utilized (Sueoka and Cheng, 1962). These authors found that it was possible to separate native DNA from denatured DNA using the stepwise elution pattern.

3. Methods of Determination of Base Composition of DNA

The first method developed for determining the base composition of DNA was the chemical analysis of the nucleotides present in an acid hydrolyzed sample of DNA. Liberation of the bases from DNA requires digestion of DNA in 70% perchloric acid at 100°C (Marshak and Vogel, 1951) or in anhydrous formic acid at 175°C (Vischer and Chargoff, 1948). The hydrolysates are then separated into the individual nucleotides by means of paper chromatography (Bendich, 1957). The bases can then be quantitated spectrophotometrically by measuring the extinction of each base at specified wavelengths. Other methods used to separate the hydrolysates into bases include ion exchange chromatography (Cohn, 1957) and paper electrophoresis (Smith, 1967).

Problems associated with hydrolysis of DNA in concentrated acids include deamination of guanine and adenine (Abrams, 1951), as well as formation of charcoals from deoxyribose and any contaminating polysaccharides, which can then adsorb the free bases leading to false results (Kirk, 1967). Kirk (1967) developed a method of determining the base composition of DNA by determination of the guanine/adenine ratio. The method involved hydrolysis of DNA in 0.03 M HCl at 100°C for forty minutes. This resulted in a quantitative release of the purine bases. After hydrolysis, the apurinic acids were removed from adenine and guanine and collected as single fractions by cation exchange chromatography. The amount of each base present was determined by measuring the volume of each fraction and the extinction at suitable wavelengths. The basis of this method is the fact that double-stranded DNA contains an equal number of adenine and thymine residues and that the number of guanine residues equals the number of cytosine (or cytosine plus 5-methylcytosine) residues. The guanine/adenine ratio is then expressed as mole per cent guanine plus cytosine in order to compare this method to other methods used for the analysis of base composition.

Broughton et al. (1972) reported several methods of DNA preparation from various sources for use with the base ratio determination procedure of Kirk (1967). Broughton et al. also modified the method of hydrolysis of DNA by using 0.1 M HCl at 100°C for forty minutes. This enabled them to determine the base ratios of unpurified DNA samples.

The base composition of DNA can also be determined by calculating the buoyant density via ultracentrifugation as developed by Meselson et al. (1957). Schildkraut et al. (1962) carried out a comprehensive

study of the relationship between the buoyant density of DNA in cesium chloride and its' base composition. A formula was derived which related buoyant density in cesium chloride to the guanine-cytosine content of DNA, based on a study of many natural and synthetic DNA samples. That a linear relationship exists between the guanine-cytosine content of DNA and buoyant density in cesium chloride, was established by Sueoka (1959), Sueoka et al. (1959), and Rolfe and Meselson (1959).

The guanine-cytosine content of DNA was related to the denaturation temperature of DNA by Marmur and Doty (1959). A linear relationship between melting temperature and guanine-cytosine content existed over a small range of guanine-cytosine contents. The thermal stability of DNA increased with increasing guanine-cytosine contents. The melting temperature values were obtained by following the optical density at 260 nm of a solution of DNA in a buffer, as a function of increasing temperature. As the DNA denatured, the optical density increased, giving rise to a sigmoidal denaturation curve. The melting temperature was taken as the temperature corresponding to one-half of the final increase in absorbance as a result of thermal denaturation.

Further research by Marmur and Doty (1962) revealed that there is a linear relationship between the melting temperature of DNA and guanine-cytosine content between 25 and 75 mole per cent guanine-cytosine. A relationship was established whereby the guanine-cytosine content of a DNA sample could be calculated from the melting temperature of the sample when the denaturation was carried out in a solvent 0.2 molar in sodium.

These same authors also found that the melting temperature of

DNA was dependent on the ionic strength of the solution used. As a given DNA sample was melted in the presence of potassium chloride ranging in concentration from 0.01 to 1.0 molar, the melting temperature increased while the transition breadth decreased. This phenomenon was attributed to less screening of the repulsive forces between the regularly spaced phosphate groups on the DNA strands, in the presence of smaller concentrations of the cation (Schildkraut and Lifson, 1965). Thus at low cation concentrations the thermal energy required to overcome the hydrogen bonding and other attractive forces between the bases was supplemented by the increased repulsive force between the phosphate groups, leading to a lowering of the melting temperature. At cation concentrations above 1 molar, a decrease in the melting temperature of all DNA was attributed to the displacement of DNA-bound water by the cations, as the hydration of DNA may be responsible for the stability of the double helix in solution (Gruenwedel et al., 1971).

Marmur and Doty (1962) also found that the melting temperature of a DNA was not affected by the molecular size. Sheared DNA having a molecular weight of about one-half that of high molecular weight DNA, produced the same melting curve and melting temperature as the high molecular weight DNA.

The three methods of determining base composition have been compared by various workers. A comparison of base composition obtained by paper chromatographic analysis of DNA hydrolyzed in 70% perchloric acid, and by thermal denaturation was made by DeLey and Schell (1963). Results indicated that the thermal denaturation method yielded more reproducible results. Gasser and Mandell (1968) established that a

linear relationship exists between the guanine-cytosine content of DNA as derived by buoyant density analysis and the guanine-cytosine content as determined by chemical analysis. Their work centred on bacteria of the Lactobacillus species. Mandel et al. (1970) established that there is a linear relationship between guanine-cytosine contents of bacterial DNA as determined by cesium chloride buoyant density analysis and melting temperature analysis.

DeLey (1970) examined the association between the three methods of determining the base composition of DNA. It was found that the relationships established are valid for nonbacterial DNA as well as for bacterial DNA. Unusual bases present in nonbacterial DNA probably account for the greater degree of scatter observed. Thermal denaturation temperatures were found to be affected very little by the substitution of hydroxymethylcytosine for cytosine. Buoyant density values, however are known to be affected by substitution of rare bases for one of the standard bases in DNA.

4. Base Composition of Wheat DNA

Base composition of DNA appears to be of value in the taxonomic classification of microorganisms (Mandel, 1969; Nakase and Komagata, 1970). Bendich and McCarthy (1970b) measured the relationships among the deoxyribonucleic acids of the three genomes comprising hexaploid wheat. It was found that if any difference in base composition between hexaploid and tetraploid wheats existed, it was too small to be measured by the buoyant density gradient method in cesium chloride.

Bendich and McCarthy (1970a) reported the guanine-cytosine content of hexaploid wheat as being 39 mole per cent as calculated by

thermal denaturation in 0.2 molar sodium and 43 mole per cent as determined by buoyant density. The chemically determined base composition of wheat germ DNA was reported as 47 mole per cent guanine-cytosine by Schildkraut et al. (1962). Spencer and Chargaff (1963) reported the guanine-cytosine content of wheat germ DNA to be 44.5 mole per cent as determined by chemical analysis. They reported the 5-methylcytosine content to be 5.6 mole per cent. A value of 46.0 mole per cent guanine-cytosine content for wheat germ DNA as determined by melting temperature analysis, was reported by Marmur and Doty (1962).

There appears to be a difference between the guanine-cytosine content of wheat germ DNA and wheat leaf DNA. Chen and Osborne (1970) reported melting temperatures of 86.5 to 87 °C for wheat embryo DNA and 84.5 °C for germinated wheat embryo DNA, indicating that there was a modification of the DNA involved with germination. The lower melting temperature of DNA from germinated embryo was interpreted as a deletion of guanine-cytosine rich regions of the genome during early stages of germination.

III. MATERIALS AND METHODS

A. CHEMICALS

Sodium chloride, urea, disodium phosphate, monosodium phosphate, mercuric oxide, boric acid, trisodium citrate, ammonium persulfate, Bromphenol blue, and sodium borate decahydrate were certified A.C.S. reagents. Glycine and sodium cacodylate were of reagent grade. Phenol, sodium dodecyl sulfate and bentonite were of laboratory grade. Tris(hydroxymethyl)aminomethane (Tris) was obtained as Certified Primary Standard. All of the above reagents, including Hyflo Supercel, were obtained from Fisher Scientific Company, Forest Lawn, New Jersey. Disodium(ethylenedinitrilo)tetra acetate and potassium chloride were obtained as "Baker Analyzed" reagents from the J. T. Baker Co., Philipsburg, N. J. Methyl red was obtained from Allied Chemicals, Morristown, New Jersey. Methylene blue (chloride salt) was obtained from Hartman-Reddon Company, Philadelphia, Pa. Acrylamide, N-methylenebisacrylamide, N,N,N',N'-tetramethylenediamine and 2-mercaptoethanol were obtained from Eastman Kodak Chemicals, Distillation Products Industries, Rochester, New York. Coomassie brilliant blue, myoglobin (sperm whale, salt free), chymotrypsinogen A (beef pancreas, 5 x crystallized, salt free), ovalbumin (2 x crystallized), and albumin (bovine, crystallized) were obtained from Schwarz / Mann, Orangeburg, New York. Aldolase was obtained from Pharmacia Fine Chemicals, Piscataway, New York. Albumin, Cohn Fraction V (bovine), and methylated bovine albumin were obtained from Nutritional

Biochemicals, Cleveland, Ohio. Pronase, B grade (activity 45,000 P.U.K. per g) and ribonuclease, A grade (bovine pancreas, 5 x crystallized, salt free, with a listed activity of 65 Kunitz units per mg) were purchased from Calbiochem, Los Angeles California. Most other chemicals used were of analytical grade obtained from local suppliers.

B. EQUIPMENT

Wheat was milled on a Buhler Laboratory Flour Mill, Model MLU-2, manufactured by Buhler Brothers, Uzwil, Switzerland. Thick-walled glass and stainless steel centrifuge tubes, as well as the Sorvall SS-1 Superspeed Angle Centrifuge was from the Ivan Sorvall Co. Inc., Norwalk, Connecticut. Freeze drying of flour protein extracts was carried out using a RePP (Research equipment for Pilot and Production) freeze drier, manufactured by the VirTis Co. Inc., Gardiner, N.Y. All incubations of proteins for electrophoresis and of pronase and ribonuclease solutions were carried out in a Tecan aluminum solid state Dri Block DB-3, obtained from Techne Cambridge Limited, Dunford Cambridge, England. Electrophoresis was carried out in a plexiglass cell with power being supplied by a Buchler constant current d.c. power supply, manufactured by Buchler Instruments, Fort Lee, New Jersey. Electrophoretic destaining of polyacrylamide gels was carried out with a Canalco Quick Gel Destainer manufactured by Canalco, Rockville, Md. A set of Tyler sieves used to isolate the wheat germ fraction from the shorts obtained from milling of wheat was shaken on a Tyler Portable Sieve Shaker. The sieves and shaker were manufactured by the W. S. Tyler Company of Canada Ltd., St. Catharines Ont. The mechanical

shaker used during nucleic acid extraction procedures was an Eberbach Rotator obtained from the Eberbach Corporation, Ann Arbor, Michigan. Dialysis tubing, seamless cellulose with a pore diameter of 4.8 nm retaining molecular weights of 12,000 and higher, having diameters of 0.22 and 1.125 inches (inflated) was obtained from Fisher Scientific Company Ltd., Pittsburg, Pa. Incubation of nucleic acid solutions with pronase and ribonuclease was carried out in a Fisher Serological Bath obtained from Fisher Scientific Company. A Beckman Expandomatic SS-2 pH meter, manufactured by Beckman Instruments, Inc., Fullerton, California, was used for all pH measurements. The growth chamber used for germination and growth of wheat was a Fleming-Pedlar Coldstream, Model 2-300, modified. Temperature control, for column chromatography on methylated albumin on kieselguhr was achieved with a Lo-Temptrol 154 constant temperature bath, equipped with a circulating pump. The temperature range of the bath was -10 to +10°C and it was manufactured by Precision Scientific Company, Chicago, Illinois. An LKB Perspex Peristaltic Pump manufactured by LKB Produkter, A.B., Sweden was used to maintain constant flow rates of the eluants during column chromatography. Fractions from column chromatography were collected with an ISCO model 327 fraction collector, from Instrument Specialties Company, Inc. Lincoln, Nebraska. Spectrophotometric measurements were made with a Unicam SP-1800 Ultraviolet Spectrophotometer and Unicam AR25 Linear Recorder manufactured by Pye Unicam Ltd., Cambridge England. Cuvettes, 1 cm by 1 cm of 4 ml capacity, were constructed from Spectrosil having transparency of 165 nm to 2 microns. The cuvettes used for melting temperature analysis of deoxyribonucleic acid were fitted with

teflon stoppers. These cuvettes were manufactured by Thermal Syndicate Ltd., Wallsend Northumberland, England. Temperature measurements in conjunction with melting temperature analysis experiments were made with copper-constantan thermocouples inserted in a stoppered cuvette. Voltages produced by the thermocouples were measured with a Honeywell Electronik-19 recorder manufactured by Honeywell, Ft. Washington, Pa. Temperature control during melting temperature studies was achieved with a Haake temperature controller "Thermistor", model TP32 and programmed temperature change was achieved with a Haake PG-11 temperature programmer. The bath was equipped with a R-23 pump unit capable of flow rates of 25 l / min. Temperature range of the system was -100 to +350°C and ethylene glycol was used as the heating medium. This system was manufactured by Gebruder Haake, Berlin-Lichterfelde, West Germany.

C. WHEAT SAMPLES

Three varieties of Triticum vulgare: Park, Neepawa, and Thatcher were obtained from the Alberta Wheat Pool. The crop year of each variety was 1970, 1970, and 1969 respectively. All varieties were graded as Foundation No. 1, and were stored at 4°C until used.

D. METHODS

1. Milling

The moisture content of each variety was determined by heating at 130°C for one hour using a 2 g sample of wheat ground in a Wiley Laboratory Mill to pass through a 20 mesh screen (American Association of Cereal Chemist's Laboratory Methods 44-15, 1962). Prior to milling,

the moisture content of the wheat was raised to 16.5% by tempering for 18 h at room temperature.

Milling was done on a Buhler Laboratory Flour Mill, Model MLU-2. Prior to milling the main sample of wheat, a 500 g portion of the tempered wheat, was milled in order to adjust the mill settings to obtain the flour yields desired and to clean the mill. After removing the excess flour from the mill, a known quantity of the sample was milled, the flour collected from the breaking rolls, and the flour originating from the reduction rolls was separately combined. The yield and moisture content of these samples was then determined. Weights of the shorts and bran collected were also recorded in order to determine the flour extraction percentage. The flour and shorts were separately packed into tightly closed containers and stored at 4°C.

2. Protein Extraction from the Flour Samples

a. Proteins Soluble in 0.05 N Acetic Acid

Flour (75 g) was mixed in a Waring blendor with 300 ml of 0.05 N acetic acid at 4°C for 10 min. The slurry obtained was centrifuged for 10 min at 5000 x g using a Sorvall SS-1 centrifuge. The supernatant was then decanted and quickly frozen in a 75 x 150 cm crystallization dish immersed in an acetone-dry ice bath, to a depth of 1 cm. Prior to freeze drying, the frozen samples were stored at -20°C. Freeze drying was achieved in a VirTis pilot plant freeze drier using a shelf temperature of 21°C. The dried samples were finally transferred to dry "powder" type bottles and stored at 4°C.

b. Proteins Soluble in Water, 0.4 M Sodium Chloride and 70% Ethanol

One hundred g of flour was defatted by refluxing for five min,twice, with 300 ml of anhydrous diethyl ether. The extract and the flour were separated using a Buchner funnel and vacuum filtration. Excess ether was removed in a vacuum desicator overnight at room temperature.

Flour, 75 g, was extracted three times with 300 ml of deionized water at 4°C in a Waring blendor as mentioned previously. The supernatants obtained by centrifugation at 5000 xg from the first two extractions were combined and quickly frozen for the freeze drying step.

The residue after the third water extraction was blended with 300 ml of 0.4 M NaCl-0.005 M sodium phosphate buffer, pH 7.6. This step was carried out three times. The supernatants obtained by centrifugation at 5000 xg from the first two extractions were combined and dialyzed against thirteen volumes of 0.01 N acetic acid at 4°C for 24 h with several changes of the acid during the dialysis.

The residue remaining after the third salt extraction was blended twice with 300 ml of 70% ethanol (w/w) for ten min. The supernatants obtained by centrifugation at 5000 xg were combined and dialyzed against 0.01 N acetic acid as given above.

Following dialysis, the protein extracts were quickly frozen in their respective crystallization dishes. The water, salt, and ethanol extracts were then freeze dried and stored as mentioned for the proteins extractable in 0.05 N acetic acid.

The above extraction procedures were followed for flour

collected from the breaking rolls and from the reduction rolls for each variety. Protein contents of the flours and the extracted protein samples were determined by the micro-Kjeldahl procedure using 25 mg of samples except for protein samples soluble in 70% ethanol when only 20 mg of protein sample was used. The digestion was carried out for 3 h with 2 ml of conc. sulfuric acid using mercuric oxide as the catalyst. The ammonia distillation was performed in a microscale Parnas-Wagner distillation unit trapping the ammonia in a 4% boric acid solution. The titration of this solution was then carried out with 0.02 N hydrochloric acid using a methyl red-methylene blue indicator. The acid used for titration was standardized against sodium borate, A.R. Total nitrogen values were converted to protein using the conversion factor of 5.7.

3. Polyacrylamide Disc Gel Electrophoresis of the Protein Samples

a. Electrophoresis in the Presence of 3 M Urea

Electrophoresis was carried out using 7% polyacrylamide gels containing 3 M urea. The gels were buffered to pH 8.9 by 0.38 M Tris-0.058 M HCl. The setting of the gels was performed with freshly prepared 0.28% ammonium persulfate as catalyst in electrophoresis tubes, 13 cm x 0.6 cm i.d., which were previously soaked in a dilute solution of Photo flo detergent. The gel solution added to each tube amounted to 2.5 ml and was subsequently layered with deionized water as recommended, for the polymerization step. After polymerization, the water layer was drawn off the gel surface, and the tubes were placed in the electrophoresis cell. The lower electolyte consisted of 0.005 M Tris-0.038 M glycine, while the upper electrolyte consisted of 0.012 M Tris-0.096 M glycine.

A sample of lyophilized protein was dissolved in 0.5 ml of a freshly prepared solution of 0.012 M Tris-0.096 M glycine containing 6 M urea to give a final protein concentration of 4 mg / ml. To such a protein solution, 0.5 ml of 60% glycerol was added, the solution mixed and, using a microsyringe, 60 microliters of sample was layered under the buffer on to the gel surface.

Electrophoresis was carried out in duplicate using a current of 4 mA / gel for 2 h using Bromphenol blue as the tracking dye. After electrophoresis the gels were removed from the tubes, stained for 2 h in a Coomassie blue solution consisting of 0.5 g of dye in 250 ml of solvent mixture: methanol-water-acetic acid 5:5:1 (v/v). Electrophoretic destaining of the excess dye was achieved within 20 min using a destaining solution consisting of 7.5% acetic acid and 5% methanol. Finally, destained gels were stored in 7% acetic acid.

The mobility of the protein bands was calculated relative to Bromphenol blue which was taken to have a mobility value of 1.0. The Bromphenol blue was not completely removed by staining and destaining, so any swelling of the gels due to the presence of methanol was corrected for, assuming that the swelling was even over the entire length of the disc gels.

b. Electrophoresis of Proteins Treated with Sodium Dodecyl Sulfate

For this procedure 7% polyacrylamide gels were used, buffered in 0.07 M disodium phosphate-0.027 M monosodium phosphate which contained 0.1% sodium dodecyl sulfate. The electrode chambers contained the electrolyte buffer made up of 0.14 M disodium phosphate, 0.056 M

monosodium phosphate and 0.1% sodium dodecyl sulfate. The gels were set with a 1% freshly prepared solution of ammonium persulfate as catalyst in the way described for gels incorporating urea.

A sample of lyophylyzed protein to give a final concentration of 4 mg protein / ml was dissolved in 1 ml of 0.1 M sodium phosphate buffer, pH 7.0, containing 2% sodium dodecyl sulfate and 2% 2-mercaptoethanol. The proteins, when dissolved, were incubated at 37°C for 2 h. Following incubation, the samples were dialyzed against 0.01 M phosphate buffer containing 0.1% sodium dodecyl sulfate and 0.1% 2-mercaptoethanol, at room temperature for 2 h.

After dialysis, 40 microliters of each sample was mixed with 5 microliters of 2-mercaptoethanol, 3 microliters of 0.05% Bromphenol blue and one drop of glycerol. This mixture was then applied to the gel by layering under the electrolyte buffer. Hence the amount of protein applied was 160 micrograms per gel. Samples were again run in duplicate.

Electrophoresis was carried out for 4 h with a current of 8 mA / gel. Following electrophoresis the gels were removed from the tubes and gel length as well as the distance of Bromphenol blue migration were measured. The gels were then stained in Coomassie blue for 2 h and destained electrophoretically for 20 min as done for the gels containing 3 M urea.

After destaining, the gel length and distance of migration of each protein band was measured. Relative mobility was calculated by using the formula:

$$\text{relative mobility} = \frac{\text{distance of protein migration}}{\text{gel length after destaining}} \times \frac{\text{gel length before destaining}}{\text{distance of dye migration}}$$

The formula takes into account the swelling effect that occurs in the gels during staining and destaining.

Calibration of the procedure for molecular weight estimations was performed with pure proteins of known molecular weight treated in exactly the same way as proteins isolated from the wheat flour The proteins used as standards are listed below.

PROTEIN	MOLECULAR WEIGHT
myoglobin	17,800
chymotrypsinogen A	25,600
aldolase	40,000
ovalbumin	45,000
albumin	67,000

A calibration curve was obtained by plotting the relative mobility of each pure protein standard as a function of the logarithm of the molecular weight (Weber and Osborn, 1969).

4. Nucleic Acid Extraction Procedures

a. Nucleic Acid Extraction from Wheat Embryos

Shorts of each variety, collected from the Buhler mill were shaken through a series of Tyler sieves ranging from 10 to 32 mesh. The portions remaining on the 14 and 28 mesh sieves were retained as these fractions contained the greatest amount of embryos. The embryos

were then defatted by refluxing for several minutes, a 50 g sample with 300 ml of chloroform-methanol mixture 2:1 (v/v), filtered through a Buchner funnel and the residual embryos repeatedly washed with excess of fresh solvent. The embryos were then air-dried overnight.

Nucleic acid extraction from embryos of each variety involved a gentle shaking of defatted embryos at 4°C with 50 ml of 80% acqueous phenol (w/w) (freshly purified by distillation) and 50 ml of 0.1 M Tris-HCl buffer, pH 7.8 for 60 min. The dense slurry obtained was centrifuged at 17,000 xg for 15 min using Sorvall stainless steel covered centrifuge tubes. The aqueous supernatant collected was transferred to a 600 ml beaker while the cell debris and phenol layer were re-extracted with 50 ml of Tris-Cl buffer for an additional 30 min. Repeating the centrifugation step at 17,000 xg the second supernatant was combined with the supernatant from the first extraction. Then, to these extracts, 0.1 volumes of 10% sodium chloride was added and stirred thoroughly. Ethanol, (2.5 volumes) prechilled to -20 °C, was then carefully layered using a syringe, and by careful stirring of the interface with a glass rod, nucleic acids separated as a fluffy precipitate. This was left to stand at 4 °C overnight.

The supernatant was decanted and the precipitate was pelleted by centrifugation at 5,000 xg for 10 min. Traces of phenol in the pellet were removed by washing the pellet with 10 ml portions of diethyl ether. The washed pellet was then dissolved in 5 ml of 0.015 M sodium chloride-0.0015 M trisodium citrate, pH 7.0 (SSC/10) followed by raising the concentration to 0.15 M sodium chloride-0.015 M trisodium citrate, pH 7.0 (10 SSC). All these steps as well as earlier steps

were carried out at 4°C.

Pronase (2 mg / ml) was added to this solution to a concentration of 50 micrograms / ml and incubated at 37°C overnight. The bulk pronase solution was prepared by dissolving 20 mg of the enzyme in 5 ml of distilled and deionized water, followed by adjusting to pH 5.0 with 1 N hydrochloric acid. The solution was then heated to 80°C for 10 min to destroy the possible presence of contaminating deoxyribonuclease. Then the solution was cooled, re-adjusted to pH 7.0 with 1 N sodium hydroxide, and transferred to a 10 ml volumetric flask. Solid sodium chloride was then added to the pronase solution to make it 1 molar in respect to sodium chloride (Hotta and Bassel, 1965). The solution was made up to volume with distilled and deionized water, divided into 0.5 ml portions and stored at -20°C until required.

Following the pronase digestion, the nucleic acid solution was cooled to 4°C, transferred to 50 ml erlenmeyer flasks, followed by rinsing the tube with 1 ml portions of SSC. To this digest an equal volume of deproteinizing solution of chloroform-isoamyl alcohol 24:1 (v/v) was added and the mixture was gently shaken on a mechanical shaker at 4°C for 30 min.

Centrifugation of the mixture at 2000 x g using glass, Sorvall heavy-wall centrifuge tubes separated the mixture into an upper nucleic acid aqueous layer and a lower chloroform layer with protein as a precipitate at the interface. The acqueous layer was collected by Pasteur pipet and extracted again with an equal volume of chloroform-isoamyl alcohol. This process was repeated until the protein precipitate at the interface was no longer evident. The nucleic acid solution

was finally collected and stored at -4°C over a drop of chloroform until further use.

b. Isolation of DNA from Green Wheat Leaves

Wheat kernels of each variety were spread one kernel deep into a layer of vermiculite 2 inches deep using wooden flats. Then they were covered with 1.5 inches of vermiculite, packed down and immersed in tap water until the upper layer of the vermiculite just began to float. The flats were then allowed to drain and were placed in the environmental growth chamber. Conditions of germination and growth were 21°C, 60% r.h. and 1400 ft candles light intensity.

Four days after germination, the flats were removed and placed in a cold room for 30 min. The leaves were then cut into 1 cm pieces and 20 g were placed in a mortar and chilled to 0°C. To this was added 40 ml of 0.1 M Tris-Cl buffer pH 9.0, 6.0 ml of 22% sodium dodecyl sulfate and 2 ml of 4% bentonite suspension. The bentonite suspension was prepared by suspending 10 g of bentonite powder (USP grade) in 150 ml of distilled and deionized water and stirred overnight to allow complete hydration of the clay particles. It was then centrifuged at 9000 x g for 15 min and the upper light-colored layer of the pellet, free of coarse sand particles, was collected and redispersed in 50 ml of distilled and deionized water. After a homogeneous suspension was obtained, a 4 ml aliquot was transferred to a pre-weighed aluminum dish, dried at 120°C for 2 h. The increase in weight corresponded to the amount of dry bentonite present in 4 ml of suspension. Appropriate dilutions were made to bring the concentration to 4% in water. This

suspension was then stored at 4°C until used.

To the suspension in the mortar was added 68 ml of 80% acqueous phenol (freshly distilled). As the leaves were ground in the mortar with the pestle, the phenol was added in portions thus avoiding excessive frothing during grinding.

The slurry obtained was transferred to a 500 ml erlenmeyer flask and shaken gently on a mechanical shaker for 1 h. After this, the slurry was centrifuged at 17,000 x g for 20 min using Sorvall stainless steel centrifuge tubes. The acqueous layer was collected and re-extracted by shaking with an equal volume of 80% acqueous phenol and 2 ml of 4% bentonite suspension for 30 min. After repeated centrifugation at 17,000 x g for 10 min, the acqueous layer was collected and transferred to a 200 ml beaker and nucleic acids precipitated with ethanol as previously described.

Deproteinization of the nucleic acid solution with pronase and chloroform-isoamyl alcohol was followed as already described for wheat germ nucleic acids. After this step an aliquot of the nucleic acid solution was removed for its' fractionation on a chromatographic column of methylated albumin on kieselguhr (MAK).

The remaining solution fo nucleic acids was transferred to a 10 ml screw cap culture tube and pancreatic ribonuclease was added to the nucleic acid solution to a concentration of 50 micrograms / ml followed by incubation at 37°C for 30 min to degrade the ribonucleic acids (RNA) present. To avoid deoxyribonucleic acid (DNA) digestion, the enzyme ribonuclease in 0.15 M sodium chloride, pH 5.0, (2 mg / ml) was previously heated to 80°C for 10 min in order to inactivate the

the traces of contaminating deoxyribonuclease. Following ribonuclease digestion, pronase was added to a concentration of 50 micrograms / ml and incubation continued at 37°C overnight to degrade the added ribonuclease as well as the proteins released by RNA digestion.

The crude DNA solution obtained was then deproteinized with chloroform-isoamyl alcohol treatment until the interface after centrifugation was clear. Two ethanol precipitations of DNA followed, each time recovering the DNA by winding on a glass rod. The spooled DNA was washed in 70% ethanol, dissolved in SSC/10, adjusted to SSC and then dialyzed at 4°C for 48 h against 35 volumes of SSC. Purified DNA was stored in a screw cap culture tube at 4°C over a drop of chloroform.

5. Column Chromatography on Methylated Albumin on Kieselguhr

a. Column Preparation

Methylated albumin was prepared from 5 g of albumin (Cohn fraction V) suspended in 500 ml of absolute methanol. The catalyst, 4.2 ml of 12 N hydrochloric acid, was added and the mixture was then kept in the dark at 25°C for 3 days with occasional shaking. The precipitate formed was collected by centrifugation at 6000 x g for 20 min using 250 ml polyethylene centrifuge tubes. The pellet obtained was washed twice with methanol or until free of hydrochloric acid and then twice with diethyl ether by resuspending the pellet and recentrifuging. Then the pellet was exposed to air to evaporate the residual ether and then transferred to a vacuum desicator where it was kept over potassium hydroxide until dry. The pellet was then powdered by gentle grinding in a mortar with a pestle and was finally stored again over potassium hydroxide.

To prepare a three-layer column, essentially the method of Mandel and Hershey (1960) was followed. For a simplified one-layer column, a suspension of 6 g of kieselguhr in 30 ml of 0.05 M sodium phosphate buffer pH 6.7 was boiled, cooled to room temperature and then to this suspension, 1.5 ml of a 1% aqueous methylated albumin solution was added with stirring. This suspension was then poured in a water-jacketed chromatography column of 2.5 cm i.d. with a sintered glass disc which was covered with a thin layer of Whatman No. 1 cellulose powder. The suspension was allowed to settle slowly while draining the excess of phosphate buffer. Following this step 1 g of kieselguhr suspended in 1 ml of 0.2 M NaCl-0.05 M sodium phosphate, pH 6.7, was added to the top to form a mechanical protective layer. The column was then washed with 100 ml of 0.4 M NaCl-0.05M sodium phosphate, pH 6.7 (hereafter referred to as saline buffer).

b. Separation of Nucleic Acids

Nucleic acid separation was achieved by applying to the simplified one-layer column, 30 A_{260} units of wheat embryo nucleic acid. The nucleotides were washed out with 25 ml of 0.4 M saline buffer after which nucleic acids were eluted in a stepwise manner using 25 ml portions of 0.4, 0.55, 0.60, 0.62, 0.67, 0.70, 0.75, and 0.80 M saline buffers. Fractions of 5 ml were collected using the fraction collector and the absorbance of each fraction was monitored at 260 nm. The nucleic acid profile was determined by plotting the absorbance at 260 nm versus the tube number collected.

Wheat embryo nucleic acids were applied to a three-layer MAK column in an amount of 200 A_{260} units. The nucleotides were washed out with 0.3 M saline buffer after which nucleic acids were eluted

using a linear salt gradient prepared in a perspex-made gradient mixer containing 250 ml of 0.4 M saline buffer in the first and 250 ml of 1.6 M saline buffer in the second chamber. The peristaltic pump maintained through the column a constant flow rate of 0.8 ml / min. Fractions of 3 ml were collected and the absorbance monitored at 260 nm as before.

A single layer column, 14 cm in length for fractionation of both wheat embryo and wheat leaf nucleic acids was also prepared, in which case 60 A_{260} units of nucleic acids diluted in 10 ml of 0.3 M saline buffer were applied to the column. Elution was carried out at 25°C with 400 ml of saline buffer ranging from 0.4 to 1.2 M NaCl. Flow was maintained as previously stated at 0.8 ml / min with the peristaltic pump, but 2 ml fractions were collected.

The salt gradient was followed by an Abbe refractometer by taking the refractive index at 25°C of every fifth tube. Refractive index values were then converted to molarity of sodium chloride by referring to a plot of refractive index versus salt concentration at 25°C

6. Melting Temperatures of Wheat Leaf DNA

For melting temperature analysis the temperature was recorded with a copper-constantan thermocouple inserted in a stoppered cuvette of the same construction and dimensions as the sample cuvette, placed in the cell house position immediately next to the sample cuvette. The copper-constantan junction was at the centre of the cuvette. The voltage response of the thermocouple with temperature was monitored with the recorder set for a full-scale range of 2 mv. The recorder was equipped with zero suppression. Voltage readings were converted to

temperatures by referring to a chart of voltage versus temperature (°C) for the copper-constantan thermocouple, corrected for the composition of the constantan. A correction factor was determined using a hypsometer containing water, considering the barometric pressure at the time. From these values recorded with the reference junction at 0°C, the difference between the true temperature of boiling water and the temperature obtained from the recorder was obtained. This was used to correct the set of standard tables used.

For spectrophotometric measurements, samples and reference solutions were first deaerated by bubbling water vapor-saturated helium through them for 20 min. The spectrophotometer was zeroed using the solvent to be used as a blank. No correction was made for the thermal expansion of the solutions during temperature programming. The temperature recorder was zeroed before each run by inserting both thermocouples in an ice-water bath. All temperature measurements were made with the reference junction at 0°C. The thermocouple wire was taped to the cell block to prevent errors in temperature measurement due to heat loss down the thermocouple leads.

Melting temperature analyses were performed in the presence of various concentrations of sodium and potassium chlorides. For this purpose DNA stock solutions were dialyzed against 50 volumes of 0.01 N disodium(ethylenedinitrilo)tetra acetate (disodium EDTA) of pH 7.0 for 48 h during which period the buffer was changed twice daily, followed by dialysis against 50 volumes of 0.01 M NaCl in 0.005 M sodium cacodylate buffer, pH 6.25, again for 48 h with two changes of buffer daily.

Thoroughly dialyzed DNA stock solutions were then stored in 10 ml screw cap culture tubes over a drop of chloroform. Solutions of

DNA for melting temperature determinations were prepared by mixing 0.2 ml of the DNA stock solution, 0.4 ml of 0.01 M disodium EDTA-0.01 M NaCl, pH 7.01, an appropriate volume of 5 M NaCl or 4 M KCl and distilled and deionized water to make the volume to 4.2 ml. The resulting solutions had an $A_{260\ nm}$ of about 0.2. All determinations were carried out at least in duplicate. The pH of the final solutions before recording were within the range of pH 7.0 ± 0.3. These methods used were essentially as described by Gruenwedel et al. (1971). Melting temperature determinations were also performed using SSC and SSC/10 as the solvents.

The initial absorbance of the DNA solutions was determined at 25°C. The temperature in the cuvettes was then raised to within 5°C of the onset of melting and held at that temperature for about 5 min to allow the system to stabilize. Then temperature programming at 0.5°C / min was initiated and continued until the absorbance increase stopped with continued heating or until the temperature in the cuvettes was greater than 99°C at which time boiling of the solutions began.

Melting temperatures (Tm) were calculated from a plot of relative absorbance ($A_{260\ nm}$) of the sample at a given temperature t, divided by $A_{260\ nm}$ of the sample at 25°C as a function of temperature plotted at intervals of 0.5°C. The Tm reported was equal to one-half of the final increase in relative absorbance. Base composition (reflecting the mole % guanine-cytosine) of the DNA analyzed was then calculated from the formula: $(G+C) = 2.44(Tm - 81.5 - 16.6 \log M)$ where M is the molar concentration of the cation (Schildkraut and Lifson, 1965). This relationship holds true for sodium and potassium ion concentrations ranging from 0.02 to 0.20 M. The thermal transition interval, (ΔT) between 17 and 83% of the absorbance rise was calculated according to Doty et al. (1959).

IV. RESULTS

A. FLOUR MILLING AND PROTEIN EXTRACTION

The flour yield after milling for each variety was determined on the basis of the total amount of product collected. The flour extraction was 68.9%, 68.3%, and 65.6% for Park, Neepawa, and Thatcher respectively (14% m.b.). The protein contents of the flours collected are given in Table 1. Of the flour samples obtained, Thatcher flour from the breaking rolls and from the reduction rolls was lower in protein content than the other two varieties. In all cases however, the protein content of flour collected from the breaking rolls was greater than that of flour collected from the reduction rolls.

The amount of protein collected in each extraction is also given in Table 1. It appears that there is no relationship between the amount of protein extracted by each solvent used and the protein content of the flour.

B. GEL ELECTROPHORESIS OF FLOUR PROTEINS IN THE PRESENCE OF 3 M UREA

Gel electrophoresis patterns in the presence of 3 M urea of proteins extracted from flour collected from the breaking and reduction rolls are presented in Fig. 4, 5, and 6. The gels have been arbitrarily divided into three regions. The region of low electrophoretic mobility lies between relative mobilities (Rm) of 0 and 0.35. Rm values of 0.36 to 0.70 are in the region of intermediate electrophoretic mobility while the region of high electrophoretic mobility includes Rm values

Table 1

PROTEIN CONTENTS OF FLOUR AND THE PROTEIN EXTRACTS

		Breaking Rolls		
		Park	Thatcher	Neepawa
Flour	Protein (%)	15.2	12.8	15.6
	Weight Used (g)	75	50	50
	Protein in Flour (g)	11.4	6.4	7.8
Water Extracts	Protein in Extract (%)	27.8	21.3	27.6
	Weight Collected (g)	4.39	5.37	3.21
	Protein Extracted (g)	1.2	1.1	0.9
0.4 M NaCl Extracts	Protein in Extract (%)	55.0	29.7	38.3
	Weight Collected (g)	1.31	2.39	0.92
	Protein Extracted (g)	0.7	0.7	0.4
70% Ethanol Extracts	Protein in Extract (%)	79.4	81.2	78.9
	Weight Collected (g)	5.5	-[a]	2.36
	Protein in Extract (g)	4.4	-	1.9
0.5 N Acetic Acid Extracts	Protein in Extract (%)	62.7	51.0	58.9
	Weight Collected (g)	5.85	4.18	4.5
	Protein in Extract (g)	3.7	2.1	2.6
Recovery of Protein (%)	Water, NaCl, and 70% Ethanol Extracts	55.0	-	39.4
	0.5 N Acetic Acid Extracts	32.4	33.3	33.3

[a]Part of the sample was lost.

Table 1--Continued

Reduction Rolls		
Park	Thatcher	Neepawa
11.9	10.2	11.5
75	75	75
8.9	7.6	8.6
20.6	19.0	19.0
5.7	5.13	5.52
1.0	1.1	1.1
46.9	40.8	37.2
0.85	1.33	1.49
0.4	0.5	0.5
82.0	79.6	81.0
2.12	2.23	2.98
1.8	1.8	2.4
57.6	53.0	57.5
7.13	5.8	6.98
4.1	3.1	3.9
36.1	44.0	45.9
46.1	40.2	45.2

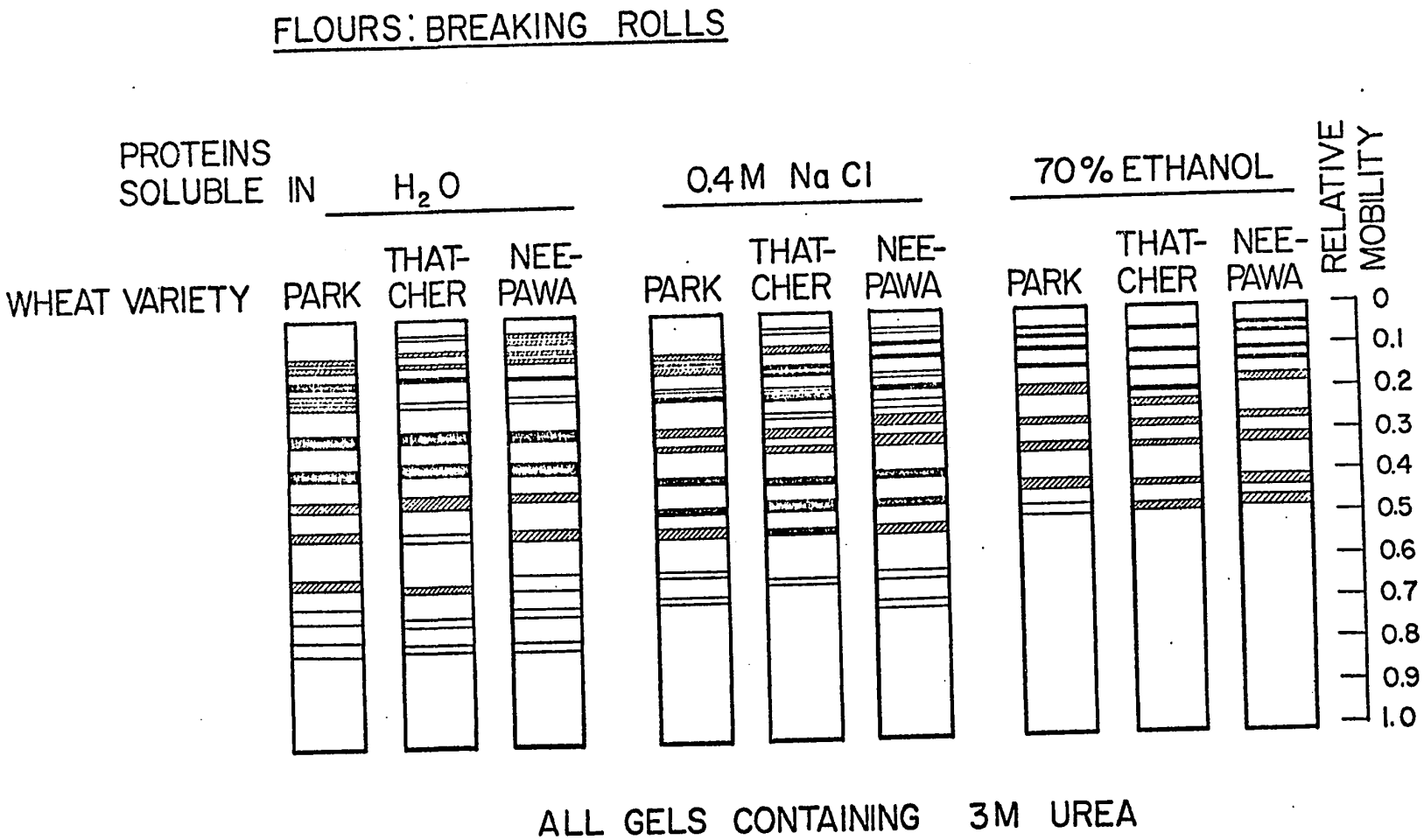


Figure 4

Electropherograms, in the Presence of 3 M Urea, of Proteins Soluble in Water, 0.4 M NaCl, and 70% Ethanol, Extracted from Flour Collected from the Breaking Rolls

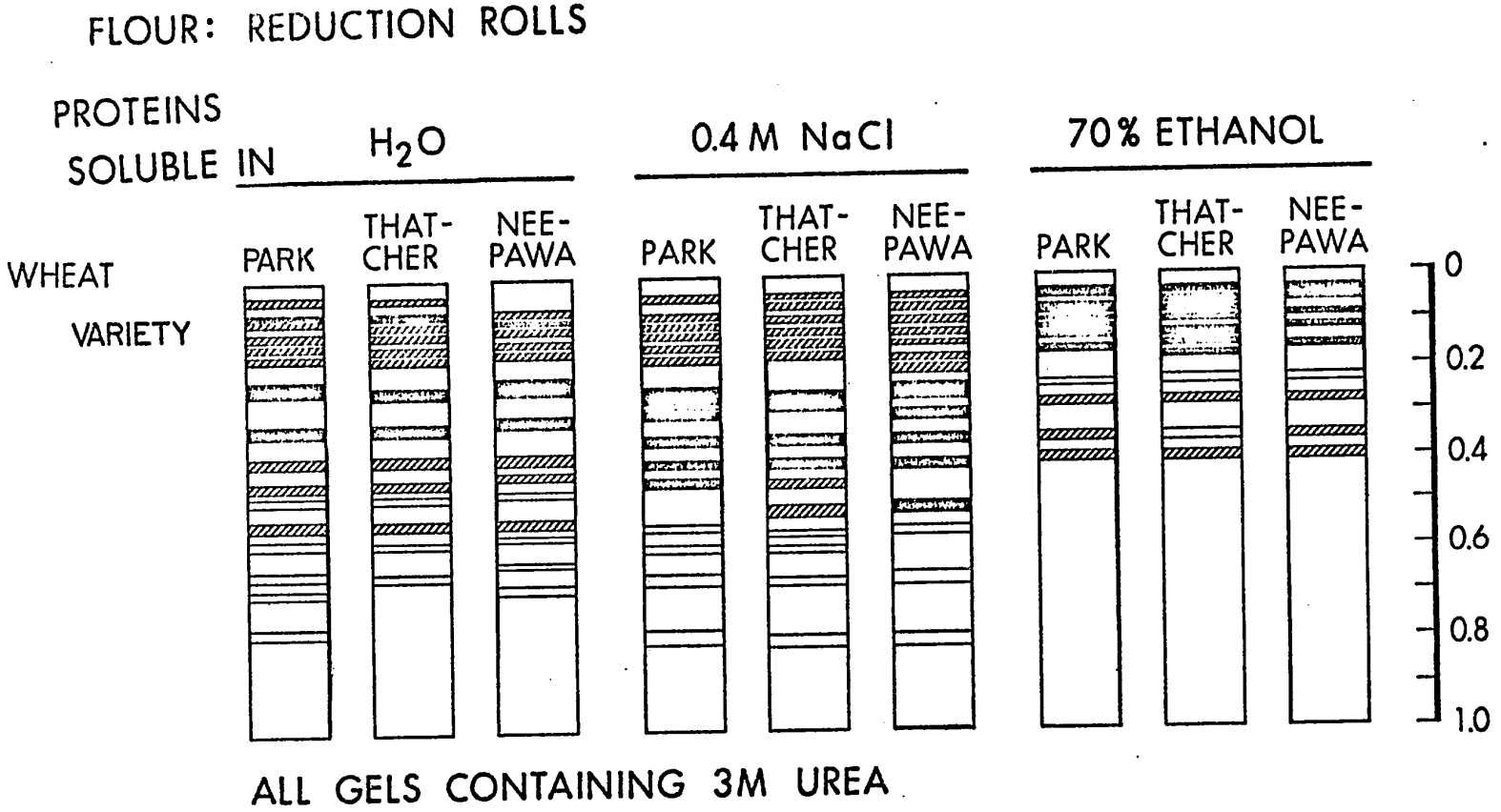


Figure 5

Electropherograms, in the Presence of 3 M Urea, of Proteins Soluble in Water, 0.4 M NaCl, and 70% Ethanol, Extracted from Flour Collected from the Reduction Rolls

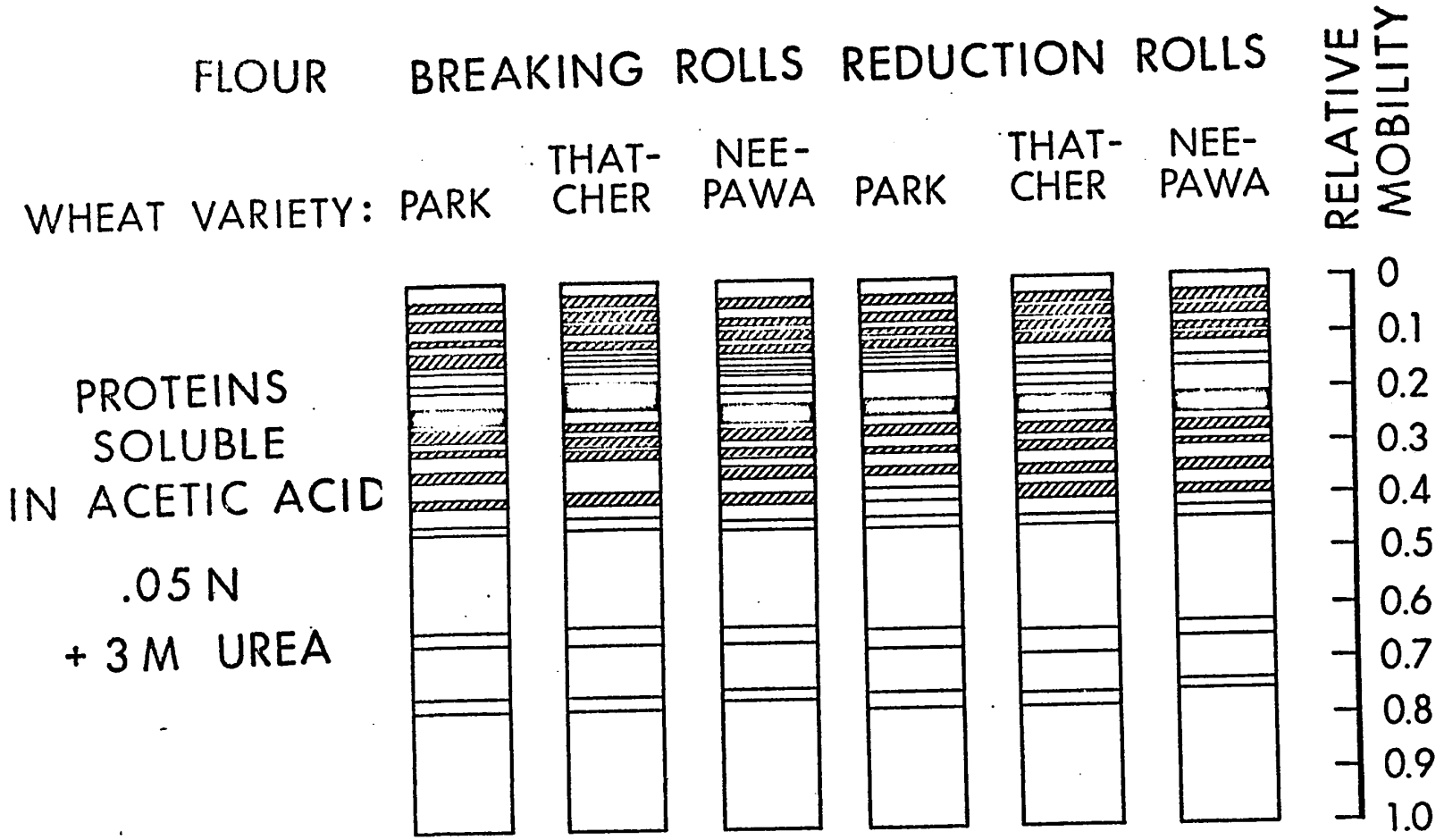


Figure 6

Electropherograms, in the Presence of 3 M Urea, of Proteins Soluble in 0.05 N Acetic Acid Extracted from Flour Collected from the Breaking Rolls and from the Reduction Rolls

of 0.71 to 1.00.

1. Proteins Soluble in Water

For water soluble proteins extracted from the flour collected from the breaking rolls, Park and Thatcher contained 12 protein bands and Neepawa contained 13 bands. In the region of low electrophoretic mobility there were 6 bands in Park, with major bands having relative mobilities of 0.15 and 0.28. Thatcher contained 6 bands in this region with 2 major bands (Rm = 0.14 and 0.28). Neepawa contained 7 bands with 2 major bands with Rm values of 0.14 and 0.28.

In the region of intermediate electrophoretic mobility all varieties contained a major band having an Rm value of 0.36. Park and Thatcher both had 4 bands in this region while Neepawa had 5 bands.

Both Park and Thatcher contained 2 faint bands in the region of high electrophoretic mobility , while Neepawa contained only one faint band.

The major bands were of the same relative mobility for all three varieties. The differences among varieties were in the region of low electrophoretic mobility where Park contained 4 medium bands (Rm = 0.09, 0.11, 0.18, and 0.20) while Thatcher contained 2 medium bands at 0.08 and 0.11 with 2 faint bands at 0.04 and 0.20. Neepawa contained 4 medium intensity bands having relative mobilities of 0.04, 0.06, 0.08, and 0.10 along with a faint band having a relative mobility of 0.19.

Electrophoresis of the water extracts of flour collected from the reduction rolls indicated the presence of a total of 16 protein bands for Park and 14 bands for Thatcher and Neepawa respectively.

Thatcher contained 8 protein bands in this region with major bands having Rm values of 0.07, 0.24, and 0.32. Neepawa contained 8 bands with 3 major bands having relative mobilities of 0.07, 0.24, and 0.32.

In the region of intermediate electrophoretic mobility, Park contained 7 bands. However, both Thatcher and Neepawa contained 6 bands. None of the bands in this region were of major intensity. The region of high electrophoretic mobility contained one faint band for Park but none for Thatcher and Neepawa.

The major bands of all three varieties had the same relative mobilities. Differences among varieties existed in the region of low electrophoretic mobility in which region Park and Thatcher contained a medium intensity band having an Rm value of 0.03 while Neepawa was lacking this band. The major band in Neepawa with Rm = 0.09 corresponded to a medium band in Park and Thatcher, while the major band at 0.07 in Park and Thatcher corresponded to a medium band in Neepawa.

In the region of intermediate electrophoretic mobility Neepawa lacked a faint band having an Rm value of 0.65, but had one at 0.62; whereas Park and Thatcher contained the former but lacked the latter band. Park and Neepawa contained a faint band having a relative mobility of 0.69, whereas Thatcher lacked this band. Park contained a faint band with Rm = 0.78, whereas both Thatcher and Neepawa lacked this band.

Although some differences existed between varieties in the electrophoretic patterns of the water soluble proteins, there was no clear-cut evidence for varietal differences. The flour collected from the reduction rolls contained more protein bands than flour collected from the breaking rolls.

2. Proteins Soluble in 0.4 M NaCl

Proteins soluble in 0.4 M NaCl from flour collected from the breaking rolls were separated into a total of 12 protein bands for Park, and 13 bands for both Thatcher and Neepawa. In the region of low electrophoretic mobility, Park contained 7 bands, of which 2 were major intensity bands having Rm values of 0.12 and 0.20. Thatcher contained 9 bands of which 3 were major ones having relative mobilities of 0.12, 0.14, and 0.20. Neepawa contained 8 bands in this region, of which 3 were major bands having Rm values of 0.08, 0.12, and 0.20.

In the region of intermediate electrophoretic mobility, Park contained 5 bands of which 2 were of major intensity with Rm values of 0.39 and 0.46. Thatcher, on the other hand, contained 4 bands of which 3 were major ones having Rm values of 0.40, 0.46, and 0.52. Neepawa contained 5 bands of which 2 were of major intensity having Rm values of 0.4 and 0.47.

In the region of high electrophoretic mobility, none of the varieties had any visible moving bands.

For this set of salt-soluble proteins, the dominant differences existed in the position of the major bands. All varieties contained major bands having Rm values of 0.12, 0.20, 0.40, and 0.47. However, Thatcher contained additional bands at 0.14 and 0.52, while Neepawa had a major band with an Rm value of 0.08. The major band having an Rm of 0.52 in Thatcher corresponded to a medium intensity band in Park and Neepawa. Neepawa and Thatcher had faint bands having Rm values of 0.04 and 0.24 while Park lacked these bands. In addition, Park lacked a band having an Rm value of 0.08 while, in this position,

Thatcher had a medium band and Neepawa, a major band. Both Park and Neepawa contained faint bands having Rm values of 0.63 and 0.70, while Thatcher lacked the band of 0.70.

For flour collected from the reduction rolls, the salt-soluble proteins of all varieties contained a total of 15 protein bands. In the region of low electrophoretic mobility, Park contained 9 bands with 3 major bands having Rm values of 0.25, 0.30, and 0.35. Thatcher contains 8 bands with 2 major bands having Rm values of 0.27 and 0.35, while Neepawa contained 10 bands with 3 major bands having Rm values of 0.25, 0.30, and 0.35.

In the region of intermediate electrophoretic mobility, Park contained 5 bands with 2 major bands having Rm values of 0.40 and 0.45, while Thatcher contained 6 bands with 1 major band having an Rm value of 0.41. Neepawa contained 4 bands with the 2 major bands having Rm values of 0.41 and 0.51.

The region of high electrophoretic mobility contained one faint band having an Rm value of 0.80, being the same for all three varieties.

The most obvious set of differences for these salt-soluble proteins were in the major bands in the low and intermediate electrophoretic regions, where Park and Neepawa contained 5 major bands, while Thatcher had only 3. Park and Neepawa had 4 of these bands in common, while 3 bands of Thatcher coincided with those of Park and Neepawa. The fast-moving major band of Park (Rm = 0.45) had the same Rm value as a medium intensity band in Thatcher. Thatcher had a medium band Rm = 0.51 which was in the same location as the fast-moving major

band of Neepawa. Each variety contained a set of medium bands near the top of the gel with Park containing 5 bands, Thatcher 6 bands, and Neepawa 7 bands.

In conclusion, for salt-soluble proteins, differences were evident between varieties but none were clear-cut. Each variety had much in common with the other 2 varieties. Flour from the breaking rolls contained fewer proteins than that from the reduction rolls, and in addition, had a slightly different pattern of electropherograms.

3. Proteins Soluble in 70% Ethanol

Proteins from flour collected from the breaking rolls and soluble in 70% ethanol, contained 9 protein bands for each variety. Park had 7 proteins in the region of low electrophoretic mobility of which 4 major bands had Rm values of 0.05, 0.07, 0.10, and 0.14. Thatcher contained 7 bands with 4 major ones having Rm values of 0.06, 0.11, 0.15, and 0.20. Neepawa also contained 7 bands of which 4 major ones had slightly different values of 0.06, 0.08, 0.12 and 0.15.

The region of intermediate electrophoretic mobility contained 2 medium intensity bands, for each variety, with Rm values practically equal.

Finally, in the region of high electrophoretic mobility none of the varieties had any detectable bands.

Differences between varieties were again evident in the region of low electrophoretic mobility. Each variety contained 4 major bands, but those of Thatcher were spread out more than those from Park and Neepawa. Thatcher had a medium band with Rm = 0.23, which was not present in either Park or Neepawa.

The flour proteins from the reduction rolls soluble in 70% ethanol showed; for Park, a total of 10 protein bands; for Thatcher, 11; and for Neepawa, 9. In the region of low electrophoretic mobility, Park contained 9 bands, of which 6 were major ones with Rm values of 0.04, 0.07, 0.09, 0.11, 0.13, and 0.16. Thatcher had 10 bands of which 7 were of major intensity having Rm values of 0.04, 0.06, 0.08, 0.10, 0.13, 0.16, and 0.18. Neepawa contained only 8 bands in this region of which 5 were major ones having Rm values of 0.04, 0.06, 0.09, 0.12, and 0.16.

In the region of intermediate electrophoretic mobility each variety contained one band having the same mobility.

Finally, in the region of high electrophoretic mobility, similar to the results for breaking rolls, none of the varieties had any detectable bands.

In conclusion, all varieties had major bands having Rm values of 0.04, and 0.16. Thatcher had a major band at Rm = 0.18, which was absent in both Park and Neepawa. A medium intensity band in Park and Neepawa with Rm = 0.35, was present only as a faint band in Thatcher. The major differences among varieties were again located in the slow-moving major intensity bands. However, differences were not clear-cut. The flour from the reduction rolls when compared to that from the breaking rolls, had similar protein patterns. Nevertheless, some quantitative differences were evident since not as many major bands were present in proteins derived from the flour of the breaking rolls.

4. Proteins Soluble in 0.05 N Acetic Acid

Flour, for each variety, collected from the breaking rolls, and extracted with 0.05 N acetic acid contained a total of 14 protein bands for Park and Thatcher and 15 bands for Neepawa. In the region of low electrophoretic mobility, Park contained 10 bands of which one was a major band with an Rm value of 0.24. Thatcher also contained 10 bands with 1 major band having an Rm value of 0.21. Neepawa variety showed 11 bands with a major band having an Rm value of 0.23.

In the region of intermediate electrophoretic mobility, each variety contained 3 bands, with no difference in their mobilities.

Finally, for all three varieties the region of high electrophoretic mobility contained only a faint band having an Rm value of 0.76.

The major differences among the varieties in the acid soluble protein fraction were in the placement of the major band and in the values of the relative mobility of medium intensity bands in the region of the low electrophoretic mobility. Above the major band, each variety contained 4 medium intensity bands. The Rm values of these bands in Thatcher and Neepawa were 0.04, 0.07, 0.10, and 0.12; and for Park 0.04, 0.07, 0.11, and 0.14. Below the major band each variety had 4 medium intensity bands, which for Park and Neepawa had Rm values of 0.28, 0.31, 0.35, and 0.40; while for Thatcher the values were 0.26, 0.29, 0.32, and 0.40. In addition, above the major band, Park contained a faint band with Rm = 0.19, Thatcher had 2 faint bands with Rm = 0.13 and 0.15, while Neepawa contained 3 faint bands with Rm = 0.15, 0.17, and 0.20.

Proteins soluble in 0.05 N acetic acid extracted from flour collected from the reduction rolls had a total of 14 bands in Park and Thatcher and 13 in Neepawa. In the region of low electrophoretic mobility, both Park and Thatcher contained 10 bands with a major band having an Rm value of 0.23. Neepawa contained 9 components with a major band having an Rm value of 0.23.

In the region of intermediate electrophoretic mobility, Park contained 2 bands while Thatcher and Neepawa contained 3 bands.

Only one faint component with Rm = 0.77 was present in the region of high electrophoretic mobility for all three varieties.

A comparison of the electropherograms described above revealed that the major bands in each variety had the same relative mobility. Thatcher and Neepawa contained a common medium intensity band with Rm = 0.40, while this band in Park was only of faint intensity. In the low electrophoretic region Park contained 2 faint bands with Rm = 0.13 and 0.16, Thatcher similarly had 2 bands with Rm = 0.15 and 0.19, while Neepawa contained 1 faint band with Rm = 0.16.

A comparison of the protein pattern for reduction rolls with those described previously for breaking rolls has shown more differences among varieties for the proteins extracted from the flour collected from the breaking rolls. However, these differences were small, hence the electropherograms among varieties were similar. The majority of the acid-soluble proteins were of low electrophoretic mobility.

C. ELECTROPHORESIS OF FLOUR PROTEINS IN THE PRESENCE OF SODIUM DODECYL SULFATE

The electrophoretic patterns for proteins treated with sodium

dodecyl sulfate and 2-mercaptoethanol are presented in Fig. 7, 8, and 9.

1. Proteins Soluble in Water

The water-soluble fraction of flour collected from the breaking rolls contained a total of 17 bands for Park, and 19 bands for Thatcher and Neepawa. Park contained 4 faint bands in the region of low electrophoretic mobility, while Thatcher and Neepawa each contained 5. All had molecular weights over 67,000.

In the region of intermediate electrophoretic mobility representing a molecular weight range of 29,000 to 67,000, Park contained 7 bands with a major one with Rm = 0.38, corresponding to a molecular weight of 62,000. Thatcher contained 8 bands with a major one also having an Rm = 0.38. Neepawa contained 9 bands with a major one with Rm = 0.36, corresponding to a molecular weight of 65,000.

In the region of high electrophoretic mobility, representing proteins with molecular weights between 17,800 and 29,000, Park and Neepawa each contained 5 bands in contrast to Thatcher which had 6 bands. The fastest moving band of each variety was one of major intensity with Rm = 0.99, while Park also contained another major band with Rm = 0.90, corresponding to a molecular weight of 17,800.

A general comparison of all electropherograms indicated that Thatcher and Neepawa contained a faint band with Rm = 0.28, which was absent in Park. Park and Thatcher both contained a medium intensity band with Rm = 0.31, present in Neepawa as a faint band. Park contained a medium intensity band, Rm = 0.40, present as a faint band in Thatcher and Neepawa. In addition, Park and Neepawa contained 1 faint band with Rm = 0.45, absent in Thatcher. All of these differences reflected the proteins with molecular weights in the range of 53,000 to greater

FLOURS: BREAKING ROLLS

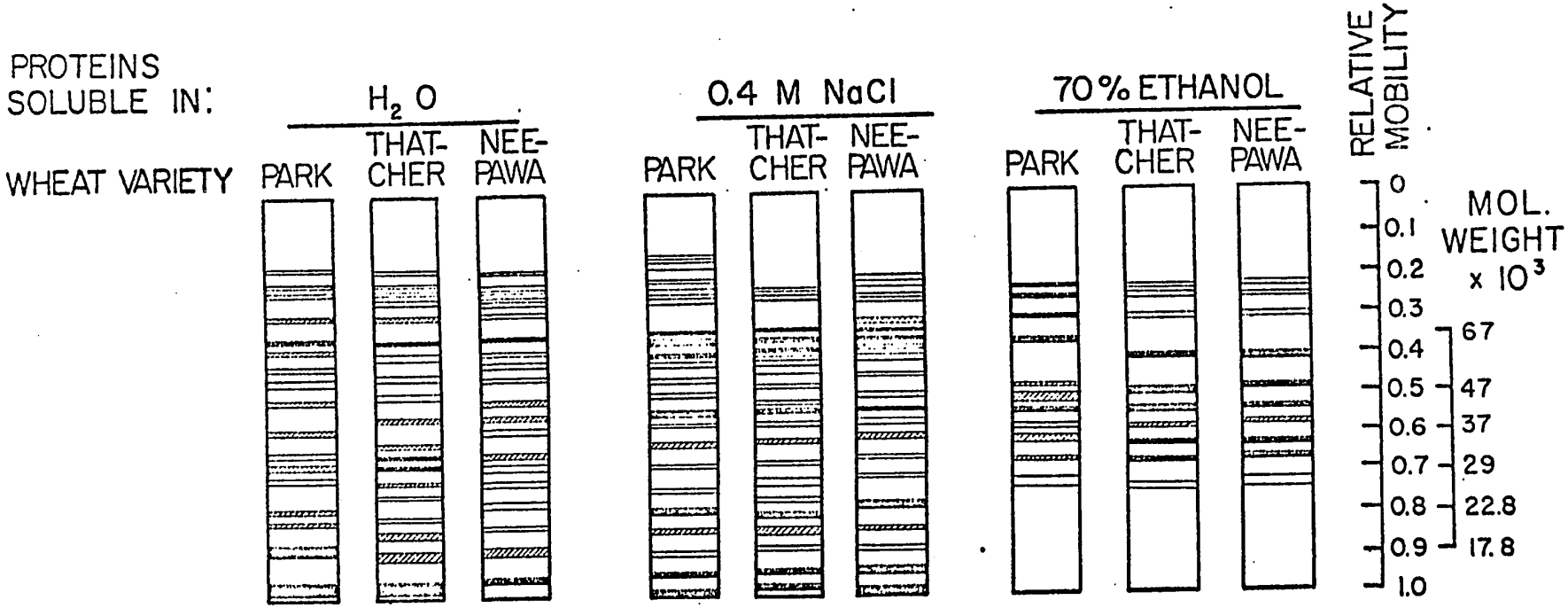


Figure 7

Electropherograms of Proteins Soluble in Water, 0.4 M NaCl and 70% Ethanol Extracted from Flour Collected from the Breaking Rolls. All Gels Contained Sodium Dodecyl Sulfate

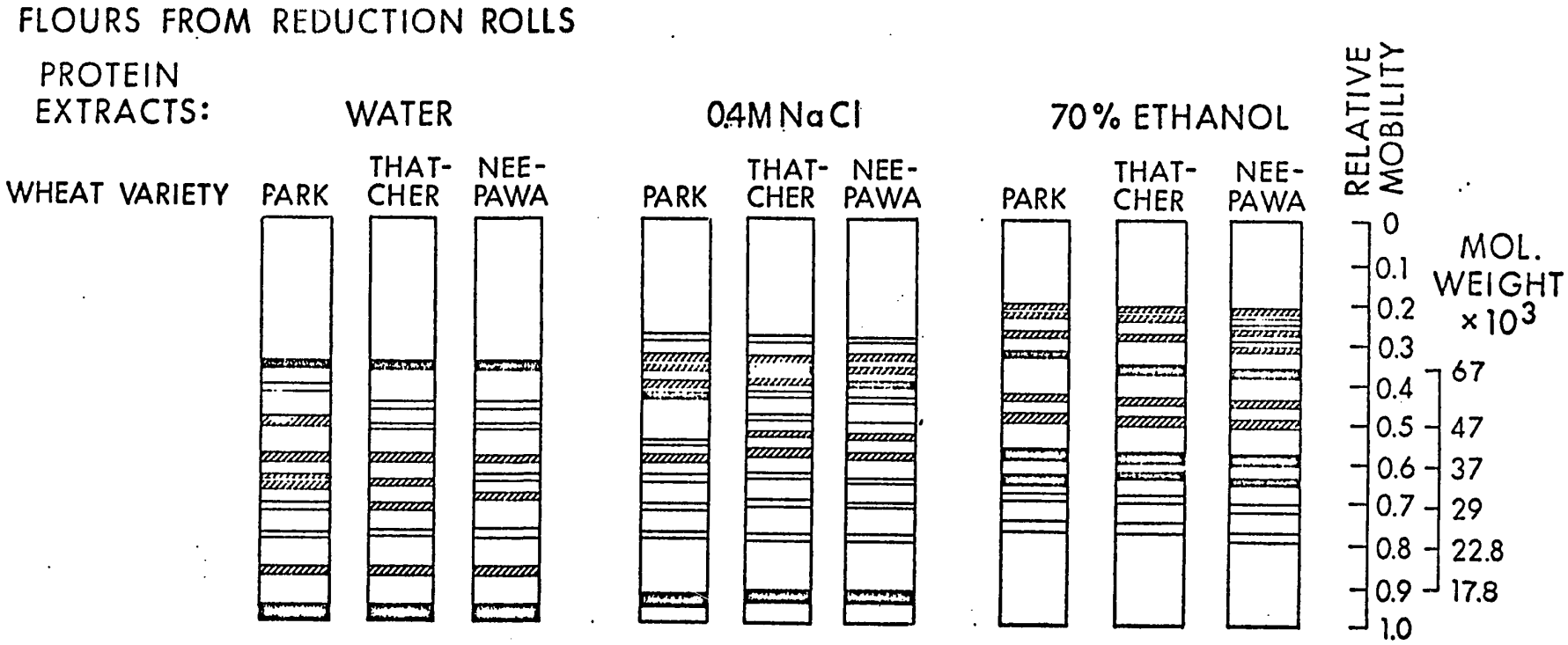


Figure 8

Electropherograms of Proteins Soluble in Water, 0.4 M NaCl and 70% Ethanol Extracted from Flour Collected from the Reduction Rolls. All Gels Contained Sodium Dodecyl Sulfate

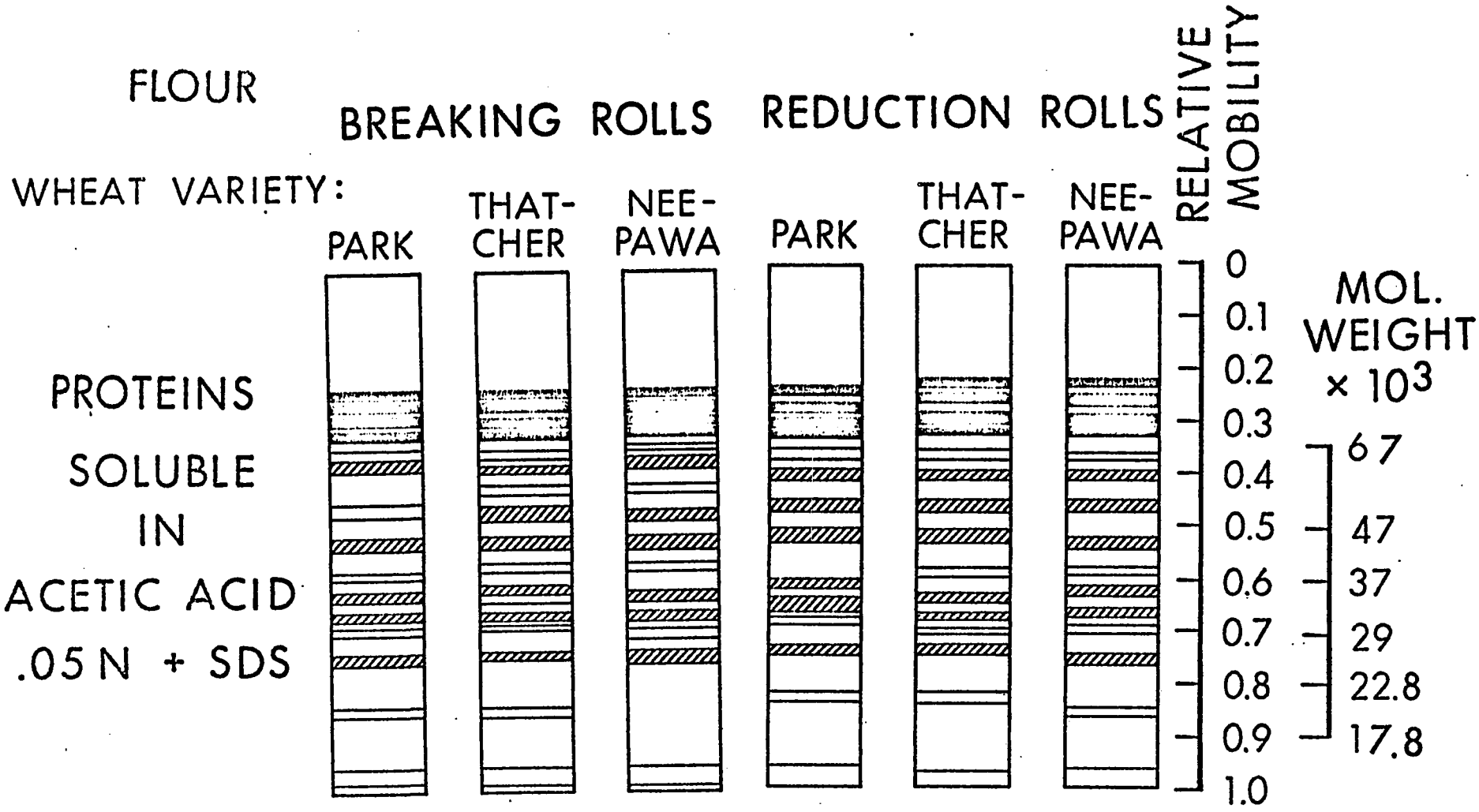


Figure 9

Electropherograms of Proteins Soluble in 0.05 N Acetic Acid Extracted from Flour Collected from the Breaking Rolls and from the Reduction Rolls. All Gels Contained Sodium Dodecyl Sulfate

than 67,000. Differences found for proteins in a range of molecular weights between 29,000 and 44,000 were as follows: Park and Neepawa contained a medium intensity band with Rm = 0.53, present as a faint band in Thatcher. Neepawa and Thatcher contained a medium band with Rm = 0.57, absent in Park. On the other hand, Park contained a medium intensity band with Rm = 0.61, present as a faint band in Neepawa and absent in Thatcher. However the latter contained a medium intensity band with Rm = 0.64, absent in Park and Neepawa. A medium intensity band with Rm = 0.67 in Neepawa was present only as a faint band in both Park and Thatcher. Park contained a medium band with Rm = 0.70, present only as a faint band in the other two varieties.

In the range of molecular weights between 17,800 and 29,000 major differences found among the protein patterns were as follows: A medium intensity band with Rm = 0.73 in Thatcher, was present as a faint band in Park and Neepawa. Park contained 2 medium intensity bands with Rm = 0.81 and 0.84, present in Thatcher and Neepawa as faint bands only. But Thatcher contained a medium band with Rm = 0.87 absent in the other two varieties. The major intensity band in Park with Rm = 0.90 corresponded to a medium band in Neepawa, while Thatcher had a medium intensity band located at 0.93.

In contrast to water-soluble proteins extracted from the flour accumulated from the breaking rolls, proteins extracted from the flour collected from the reduction rolls contained a total of 10 bands for Park, and 9 for Thatcher and Neepawa. No protein bands were detected in the range of low electrophoretic mobility, corresponding to a molecular weight range of 67,000 and over.

In the region of intermediate electrophoretic mobility, representing a range of molecular weights between 29,00 and 67,000, Park and Neepawa revealed 6 bands with a major one having an Rm = 0.36. In contrast, Thatcher had 5 bands with a major band corresponding to that of the other two varieties.

In the region of high electrophoretic mobility, representing a range of molecular weights between 17,800 and 29,000, Park and Thatcher contained 4 bands with a major one having an Rm = 0.98. Neepawa contained only 3 bands.

An overall comparison of the electropherograms revealed differences existing mainly among the bands of medium and faint intensity. Among these differences was a medium intensity band in Park with Rm = 0.43 while Thatcher and Neepawa had a faint band with Rm = 0.47. In addition, Park contained a medium band with Rm = 0.52 present as a faint band in the other two varieties. Park also contained 2 medium intensity bands with Rm values of 0.65 and 0.67, while Thatcher contained a medium intensity band at 0.67 and Neepawa had a faint band at 0.65. In addition, Neepawa contained a medium band at Rm = 0.70, while Thatcher had a medium band at Rm = 0.72 present as a faint band in Park.

In conclusion, the flour proteins from reduction rolls, when compared to those from the breaking rolls, had fewer protein bands. In addition, the reduction roll proteins did not contain several of the high molecular weight bands (Rm less than 0.35), present in flour proteins from the breaking rolls.

2. Proteins Soluble in 0.4 M NaCl

Flour from the breaking rolls extracted with 0.4 M NaCl contained a total of 21 bands for Park and 20 bands for Thatcher and Neepawa,

In the region of low electrophoretic mobility corresponding to molecular weights of 67,000 and greater, Park revealed 6 protein bands, Thatcher 4, and Neepawa 5; each having the major band at Rm = 0.34.

In the region of intermediate electrophoretic mobility, representing molecular weights in the range of 29,000 to 67,000; Park, Neepawa, and Thatcher each contained 9 bands with 3 major ones with the same Rm values of 0.36, 0.39, and 0.54.

In the region of high electrophoretic mobility in a molecular weight range of 17,800 to 29,000, Park and Neepawa both contained 6 bands while Thatcher contained 7 bands with the 3 major bands for all varieties being at Rm = 0.79, 0.95, and 0.99.

A comparison of all electropherograms obtained for this protein fraction indicated differences existing among varieties in the region of low electrophoretic mobility. Thus, Neepawa had a medium intensity band with Rm = 0.31, absent in Park and Thatcher. Also, Park contained 5 faint bands with Rm = 0.15, 0.17, 0.21, 0.23, and 0.25; while Thatcher contained two (Rm = 0.23 and 0.25), and Neepawa had 3 faint bands (Rm = 0.21, 0.24, and 0.26).

In the region of intermediate electrophoretic mobility corresponding to the range of molecular weights between 29,000 and 67,000, differences among varieties were also found, but they were restricted to faint bands. Thus, Neepawa had a band at Rm = 0.40, while Park and Thatcher had the band at Rm = 0.42. In addition, Neepawa contained the band at Rm = 0.45, while Park and Thatcher contained the band with Rm = 0.47.

In the region of high electrophoretic mobility similar differ-

ences were observed. Park contained a faint band at Rm = 0.74, while the other two varieties had the band at Rm = 0.72. Thatcher contained a band at Rm = 0.76, absent in Park and Neepawa.

For flour collected from the reduction rolls, the 0.4 M NaCl protein extracts contained a total of 11 bands for Park and 12 for Thatcher and Neepawa. In the region of low electrophoretic mobility all varieties contained 2 bands with the same mobility regardless of variety.

In the region of intermediate electrophoretic mobility, Park contained 6 bands, with the major one at Rm = 0.44; Thatcher 7 bands, the major one being at Rm = 0.38; and Neepawa 7 bands, the major one being at Rm = 0.41. These mobilities corresponded to a range of molecular weights between 54,000 and 62,000.

In the region of high electrophoretic mobility all varieties contained 3 bands with a major band at Rm = 0.94, having a molecular weight less than 17,800. No differences were detected in the band mobilities.

An overall comparison of the electropherograms of the salt-soluble protein fraction indicated major differences in the position of the major bands in the region of intermediate electrophoretic mobility. Thus, the major band of Park with Rm = 0.44 corresponded to a faint intensity band in the other two varieties. The major band in Thatcher, Rm = 0.38, corresponded to a medium intensity band in Park and Neepawa, while the major band in Neepawa at Rm = 0.44 corresponded to a medium intensity band in the other two varieties. Additionally, the medium band for Thatcher and Neepawa at Rm = 0.56, was a faint band in Park.

In conclusion, though varietal differences were detected in the salt-soluble protein fractions, they were not clear-cut. Many of the differences just reflected the quantity of the protein present in each band. As previously found, the flour from the breaking rolls contained more protein bands than that of the reduction rolls.

3. Proteins Soluble in 70% Ethanol

Ethanol-soluble proteins extracted from flour collected from the breaking rolls contained a total of 11 bands for Park, and 10 bands for each of Thatcher and Neepawa. In the region of low electrophoretic mobility, Park contained 3 bands all of which were of major intensity with Rm = 0.23, 0.26, and 0.31. In contrast, Thatcher and Neepawa contained only 3 faint bands with the same Rm values.

In the region of intermediate electrophoretic mobility, Park contained 7 bands of which 2 were major ones at Rm = 0.37 and 0.54. However, Thatcher and Neepawa both contained 6 bands of which 5 were major ones at Rm = 0.41, 0.50, 0.54, 0.63, and 0.68. Quantitative differences were also apparent in this region of electrophoretic mobility. Thus, Thatcher and Neepawa contained a medium intensity band at Rm = 0.59, which corresponded to a faint band in Park. The major band at Rm = 0.41 in Thatcher and Neepawa had no matching band in Park, while the band at Rm = 0.37 in Park was absent in the other two varieties. The major band at Rm = 0.50 in Neepawa and Thatcher appeared to be split into 2 medium intensity bands in Park having Rm values of 0.48 and 0.51. Furthermore, 2 major intensity bands in Thatcher and Neepawa with Rm values of 0.63 and 0.68 corresponded to the same bands of medium intensity in Park.

In the region of high electrophoretic mobility no bands were detected.

Ethanol-soluble proteins extracted from flour collected from the reduction rolls contained a total of 10 bands for Park and Thatcher and 11 bands for Neepawa. In the region of low electrophoretic mobility Park contained 4 bands with a major intensity band at Rm = 0.33. Thatcher contained 3 bands and Neepawa 4, none of which was of major intensity.

In the region of intermediate electrophoretic mobility with a molecular weight range of 29,000 to 67,000, Park contained 4 bands of which 2 were of major intensity, having Rm values of 0.58 and 0.65. Thatcher contained 6 bands while Neepawa contained 5. For both varieties 3 bands were of major intensity with Rm = 0.37, 0.59, and 0.63.

In the region of high electrophoretic mobility representing molecular weights ranging from 17,800 to 29,000, Neepawa contained 2 faint bands while the other two varieties contained 1 band.

A comparison of this protein fraction indicated differences among varieties in the region of low electrophoretic mobility. Park and Thatcher contained 3 medium bands having Rm values of 0.22, 0.24, and 0.28. In contrast, Neepawa had the first and third band, while the second was only a faint band. In addition, this variety had a medium intensity band at Rm = 0.32. Furthermore, Park contained a major band at Rm = 0.33 in contrast to the other two varieties having the major band at Rm = 0.37.

In conclusion, the ethanol-soluble proteins extracted from the flour fractions collected from the breaking and reduction rolls

contained nearly the same number of protein bands. However, flour proteins from the breaking rolls for all varieties contained more major bands than those from the flour collected from the reduction rolls. However among the proteins from the breaking rolls, Park differed markedly in the intensity of the protein bands when compared with the other two varieties. Proteins extracted from flour from the reduction rolls contained bands in the region of high electrophoretic mobility, whereas those extracted from flour collected from the breaking rolls did not contain these bands.

4. Proteins Soluble in 0.05 N Acetic Acid

Proteins soluble in 0.05 N acetic acid obtained from the flour collected from the breaking rolls contained a total of 16 protein bands for Thatcher, while the other two varieties each had 15. In the region of low electrophoretic mobility a molecular weight range of 67,000 and over, all varieties contained 5 bands of which 4 were of major intensity with Rm = 0.23, 0.25, 0.28, and 0.31.

In the region of intermediate electrophoretic mobility, a molecular weight range of 29,000 to 67,000, Park contained 7 bands; while the other two varieties contained 8. None of these bands were of major intensity.

The range of molecular weights of 17,800 to 29,000 represented by the region of high electrophoretic mobility, contained 2 bands for Neepawa and 3 for each of the other varieties.

A general comparison of the electropherograms of acid-soluble proteins indicated differences in medium and faint intensity bands in the regions of intermediate and high electrophoretic mobility. A faint band at Rm = 0.45 in Park was present as a medium band in the

other two varieties. In addition, a faint band at Rm = 0.41, present in the other two varieties, was absent in Park. Neepawa lacked a faint band at Rm = 0.74, which was present in Park and Thatcher.

The acid-soluble protein from the flour collected from the reduction rolls contained a total of 14 bands for Park and 15 for each of the other two varieties. In the region of low electrophoretic mobility each variety contained 4 major intensity bands at Rm = 0.22, 0.26, 0.29, and 0.32.

In the region of intermediate electrophoretic mobility with molecular weights ranging from 29,000 to 67,000, Park had 7 bands; while Thatcher and Neepawa each had 8. None were of major intensity.

In the region of high electrophoretic mobility each variety contained 3 bands, none of which were of major intensity.

A comparison revealed the absence of a faint band in Park at Rm = 0.59, present in the other varieties. In addition, a difference was found in the 2 medium bands at Rm = 0.64 and 0.67 in Thatcher which were at 0.61 and 0.65 in the other two varieties.

In conclusion, the acid-soluble proteins from breaking roll flours and from reduction roll flours, soluble in acetic acid contained a similar number of protein bands for all varieties. The position of the bands and their intensities were similar for all varieties when minor differences were neglected.

Thus, the acid-soluble proteins did not reflect any varietal differences.

5. Molecular Weight Calibration

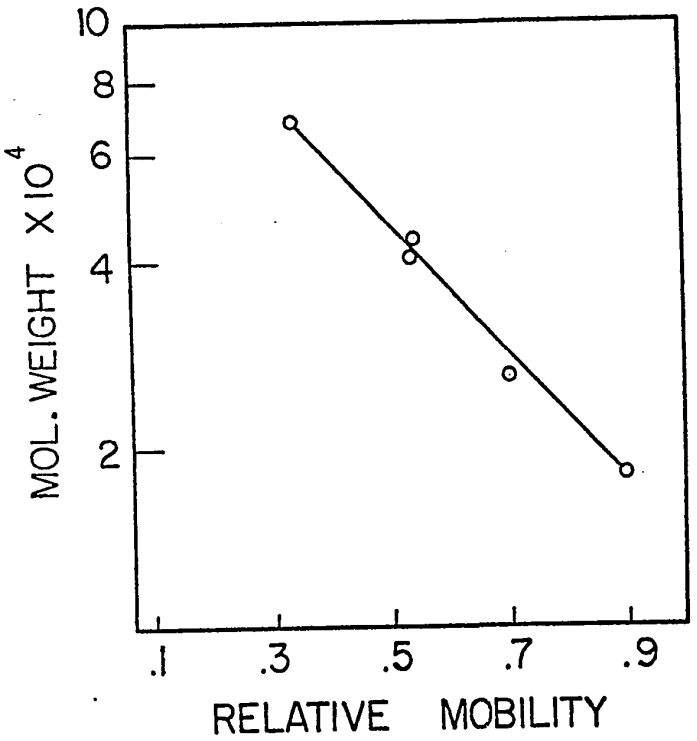
The measurement of molecular weights for all of the electro-

pherograms of proteins treated with sodium dodecyl sulfate and 2-mercaptoethanol was possible through the use of a calibration curve prepared using pure protein standards of known molecular weight. As seen in Fig. 10, the calibration curve obtained from proteins of molecular weights ranging from 17,800 to 67,000 was a straight line when the logarithm of the molecular weight was plotted against the relative mobility for each protein standard.

D. SEPARATION OF TOTAL NUCLEIC ACIDS BY MAK COLUMN CHROMATOGRAPHY

In Fig. 11, the nucleic acid profiles of several wheat varieties are presented as well as the relative composition of the individual nucleic acids collected. The DNA peak of each variety from both the embryos and the green leaves was eluted at the same concentration of sodium chloride (0.55 M). The wheat embryo DNA comprised only 5.4% of the total nucleic acids while the ribosomal RNA (rRNA) was 83% of the total nucleic acids of wheat embryos. This fraction was eluted at a sodium chloride concentration of 0.7 M. Transfer RNA (tRNA) washed free of contaminating nucleotides represented 11.4% of total nucleic acids of the embryo. The tRNA was eluted at a sodium chloride concentration of 0.45 M.

In the nucleic acid profiles of the total nucleic acid extracts obtained from green wheat leaves, a DNA content usually higher than 12% was evident. Ribosomal RNA and tRNA were present in smaller amounts when compared to the previous results for wheat embryos. Thus, due to the small yield of DNA from wheat embryo, the green leaves were selected as the source of DNA for experiments involving melting temperature



PROTEINS USED AS STANDARDS (SCHWARZ BIORESEARCH)	MOL. WEIGHT
MYOGLOBIN	17,800
CHYMOTRYPSINOGEN A	25,600
ALDOLASE	40,000
OVALBUMIN	45,000
BOVINE SERUM ALBUMIN	67,000

Figure 10

Molecular Weight Calibration Curve for Electrophoresis in the Presence of Sodium Dodecyl Sulfate

FROM LEAVES (4,5-day-old)

WHEAT VARIETY:	PARK	THATCHER	NEEPAWA	GERM
t RNA %	7.8	10	4.2	11.4
DNA %	14.1	12.1	15.3	5.4
r RNA %	78.1	77.9	80.5	83.2

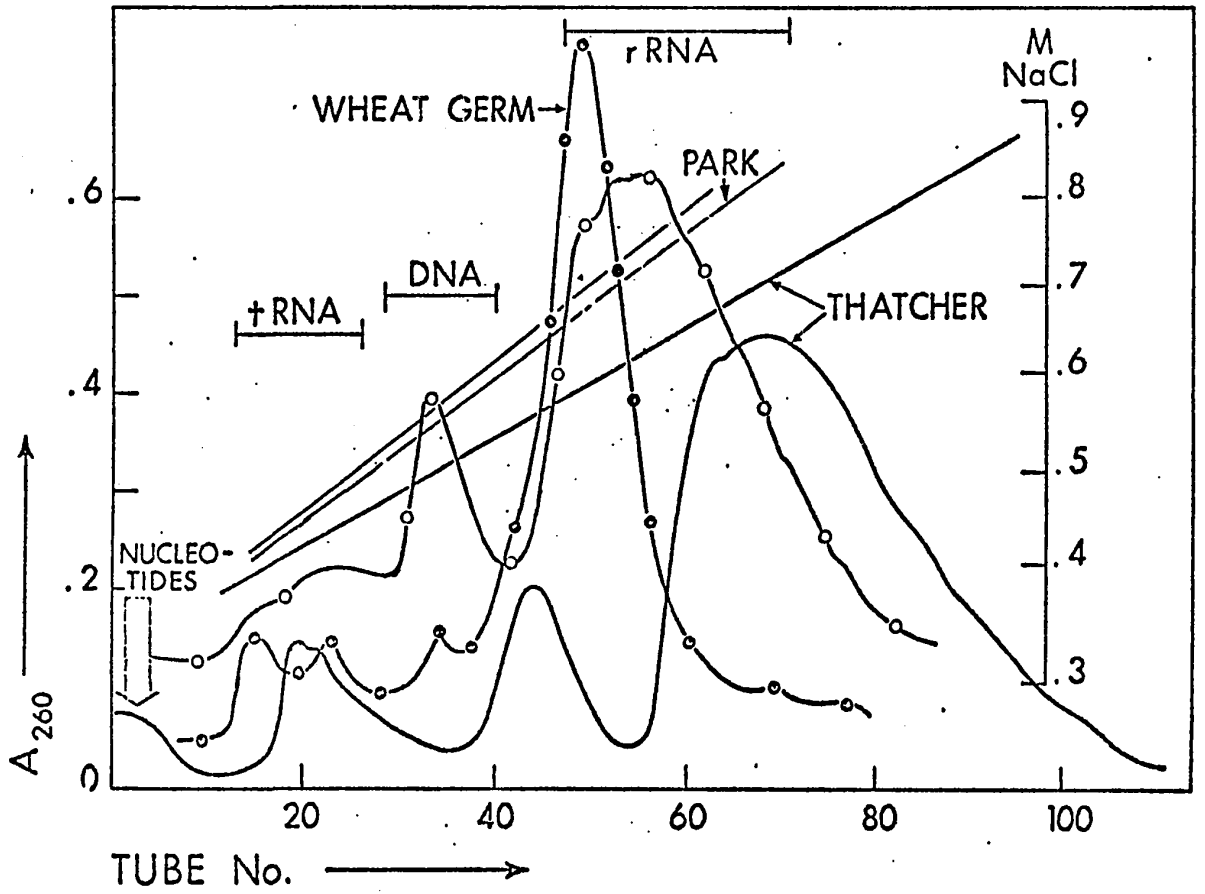


Figure 11
MAK Column Profiles of Total Nucleic Acid Extracts from Wheat Germ and from Green Wheat Leaves

determinations.

MAK column profiles of nucleic acids from Thatcher wheat shoots 4 days and 6 days old (Fig. 12) revealed that the 6 day old shoots contained more DNA and less transfer and ribosomal RNA than the 4 day old shoots. Similar results were obtained for Neepawa and Park.

E. MELTING TEMPERATURE PROFILES OF DNA

The absorbance of the DNA isolated for melting temperature studies was determined at 230, 260, and 280 nm after being purified by extensive dialysis in SSC at 4°C. The ratios of A_{230} /A_{260} and A_{280} / A_{260} for Thatcher DNA were 0.529 and 0.588; for Neepawa, 0.486 and 0.579; and for Park, 0.515 and 0.522. Additional deproteinization steps with pronase and with chloroform-isoamyl alcohol treatment did not alter the above ratios.

The melting temperature profiles of DNA from each wheat variety shown in Fig. 13 were typical of the melting temperature profiles obtained throughout this series of experiments. For the curves illustrated, the onset of melting occurred in the region of 60°C and was rapid between 70 and 80°C, tapering off after 80°C. The use of different salt concentrations altered the temperature ranges involved. In general, the curves were skewed toward the G + C rich region and in some cases small deviations from a smooth curve were observed.

During the melting temperature determination experiments the utmost care was taken to record the actual temperatures within the cuvettes. Prior to each run the temperature recorder was standardized by setting the recorder to zero electromotive force (emf) by inserting

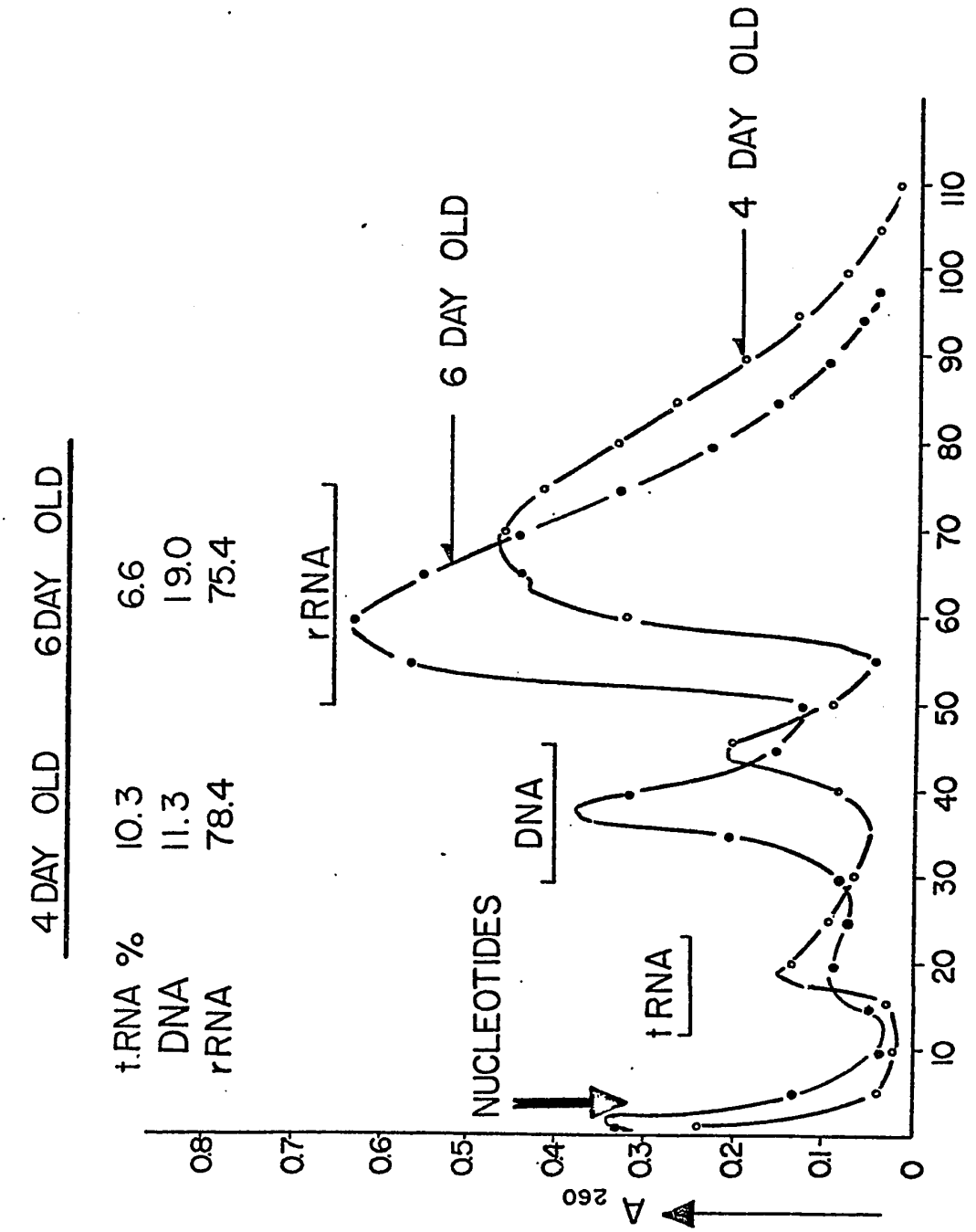


Figure 12

MAK Column Profiles of Total Nucleic Acid Extracts from 4 Day Old and from 6 Day Old Thatcher Wheat Leaves

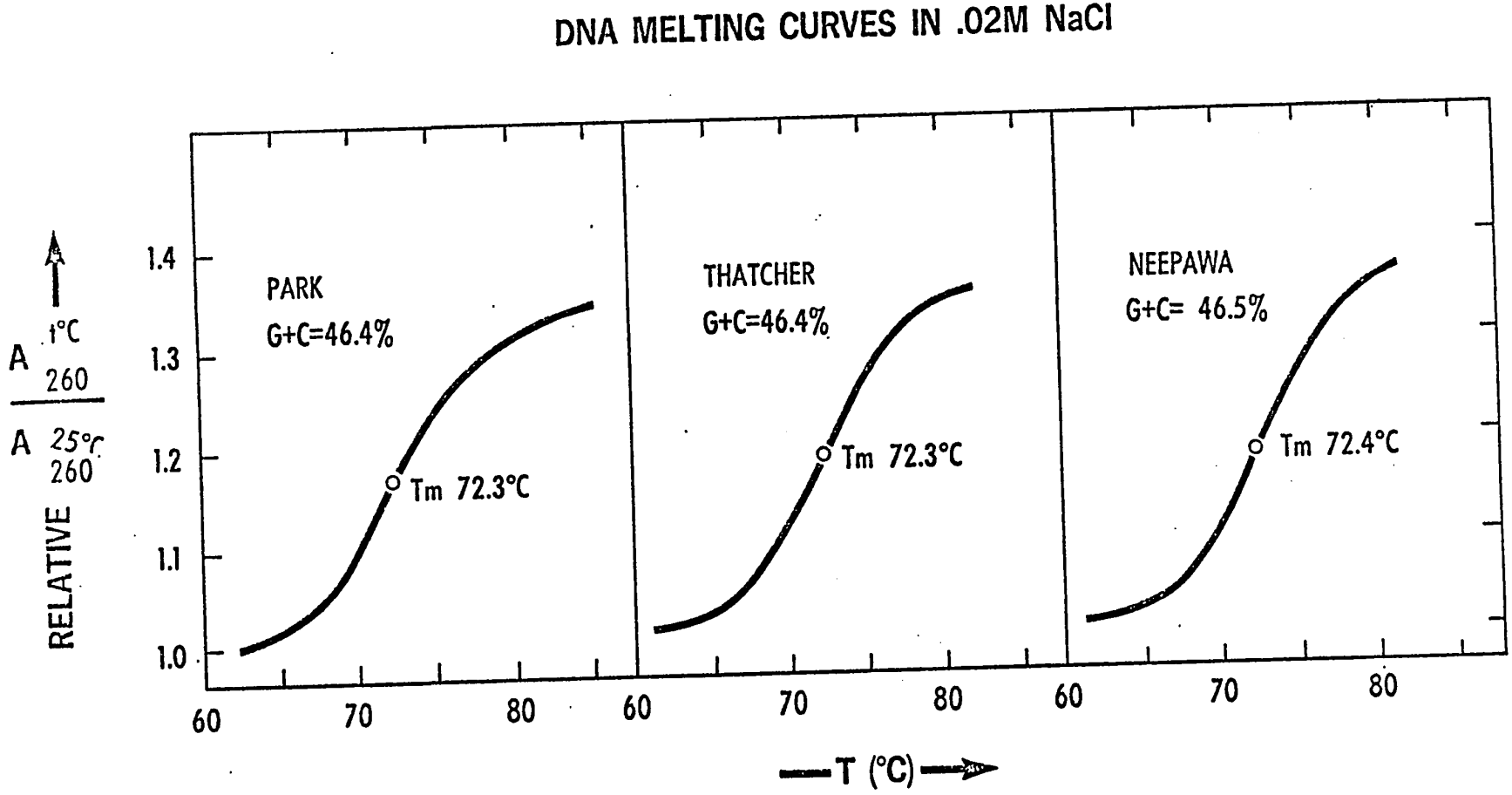


Figure 13

Melting Temperature Profiles of DNA from Park, Thatcher, and Neepawa in 0.02 M Sodium Chloride

both of the thermocouples in the ice-water bath. Care was taken to ensure that the junction of the thermocouple used for temperature measurements was placed at the centre of the cuvette.

The deviation in temperature between the sample cuvette and the cuvette used for temperature measurements was sought. To obtain this value, the temperature recorder was set at mid scale, using a full voltage range of 0.5 mv, with both thermocouples at 0°C. Following this step, a thermocouple was inserted into the sample cuvette and one was inserted into the cuvette used for temperature measurements. Both cuvettes contained deaerated SSC. Temperature increase was then programmed at 0.5°C / min from a starting temperature of 30°C. A deviation in temperature between the cuvettes was indicated by a deviation in the tracing from the zero setting where both thermocouples were of the same temperature and produced the same emf. As the maximum temperature of about 95°C in the cuvettes was achieved, the temperature in the sample cuvette was 0.5°C less than that in the cuvette used for measurement of the temperature. At lower temperatures this deviation was less than that at higher temperatures. A standard curve of cuvette temperature versus the temperature deviation could not be plotted as there was no means of determining the real temperature in the cuvettes as this operation was performed. However, this did indicate that the melting temperatures obtained in further experiments were accurate to within 0.5°C representing an uncertainty of 1% in guanine-cytosine content.

The results obtained for melting temperature determinations in various salt concentrations are presented in Table 2. These results

Table 2

EFFECT OF SALT CONCENTRATION ON THE MELTING TEMPERATURE OF DNA

Salt Concentration In Moles / 1	Salt	Wheat Variety	Tm °C	G + C Content Mole %	ΔT °C
0.005	NaCl	Park	61.1	- [a]	8.9
		Thatcher	60.8	-	9.3
		Neepawa	60.6	-	9.3
0.008	KCl	Park	65.0	-	8.8
		Thatcher	65.2	-	8.6
		Neepawa	65.0	-	9.3
0.02	NaCl	Park	72.3	46.4	9.1
		Thatcher	72.3	46.4	9.1
		Neepawa	72.4	46.5	9.7
0.02	KCl	Park	72.3	46.4	9.0
		Thatcher	72.4	46.6	9.1
		Neepawa	72.4	46.6	9.2
0.1	NaCl	Park	84.2	47.0	9.1
		Thatcher	84.3	47.3	9.2
		Neepawa	84.2	47.0	9.5
0.1	KCl	Park	84.0	46.6	9.2
		Thatcher	84.3	47.3	9.0
		Neepawa	84.4	47.6	8.7
0.2	NaCl	Park	89.0	46.6	8.5
		Thatcher	88.9	46.4	8.3
		Neepawa	89.0	46.6	8.3
0.2	KCl	Park	88.9	46.4	8.0
		Thatcher	88.8	46.2	8.1
		Neepawa	88.8	46.2	8.1
0.5	NaCl	Park	93.9	-	7.1
		Thatcher	94.0	-	6.9
		Neepawa	93.8	-	7.0
0.5	KCl	Park	93.6	-	7.0
		Thatcher	93.5	-	6.6
		Neepawa	93.2	-	6.8

[a] The (G + C) values for DNA in salt concentrations falling outside the range of 0.02 to 0.2 M could not be calculated, as the relationship between (G + C) and Tm has not been established.

revealed that as the concentration of sodium chloride or potassium chloride increased, the thermal stability of the DNA of each variety increased as shown by higher melting temperature values. In addition, where the mole per cent guanine-cytosine (G + C) content could be calculated, all of the varieties had very similar G + C contents. At the 95% confidence interval level, ($G + C \pm 2\sigma$), in NaCl solvents the G +C for Park was (46.5 ± 1.35) %. For Thatcher it was (46.8 ± 1.90) % and for Neepawa it was (46.5 ± 1.72) %. In KCl, the G + C content of each variety at the 95% confidence interval was for Park, (46.4 ± 0.74) %; Thatcher, (46.5 ± 1.57) %; and for Neepawa the value was (46.8 ± 1.09) %. Thus all varieties contained essentially the same base composition calculated as mole per cent guanine plus cytosine. The melting temperatures in SSC/10 and SSC were the same as those in 0.02 and 0.2 M sodium chloride. The hyperchromicity for all samples was between 33 and 45%, indicating that the DNA was in the native state.

The values of ΔT in Table 2 represent the temperature interval between 17 and 83% of the absorbance rise for a DNA sample, as determined from the temperature-relative absorbance curves. All varieties had essentially the same ΔT values which decreased as the salt concentration increased.

The increase in melting temperatures as a function of salt concentration was linear over the range of 0.005 M to 0.50 M of the salts used. This linearity was observed when the melting temperature was plotted against the negative logarithm of the cation concentrations expressed as moles / l (see Fig. 14). No evidence for varietal differences could be detected, again, from the melting curves of DNA. Melting

temperature determinations at greater than 0.5 M salt concentrations were beyond the scope of the equipment used due to vapor bubble formation in the stoppered cuvettes at temperatures greater than 99°C.

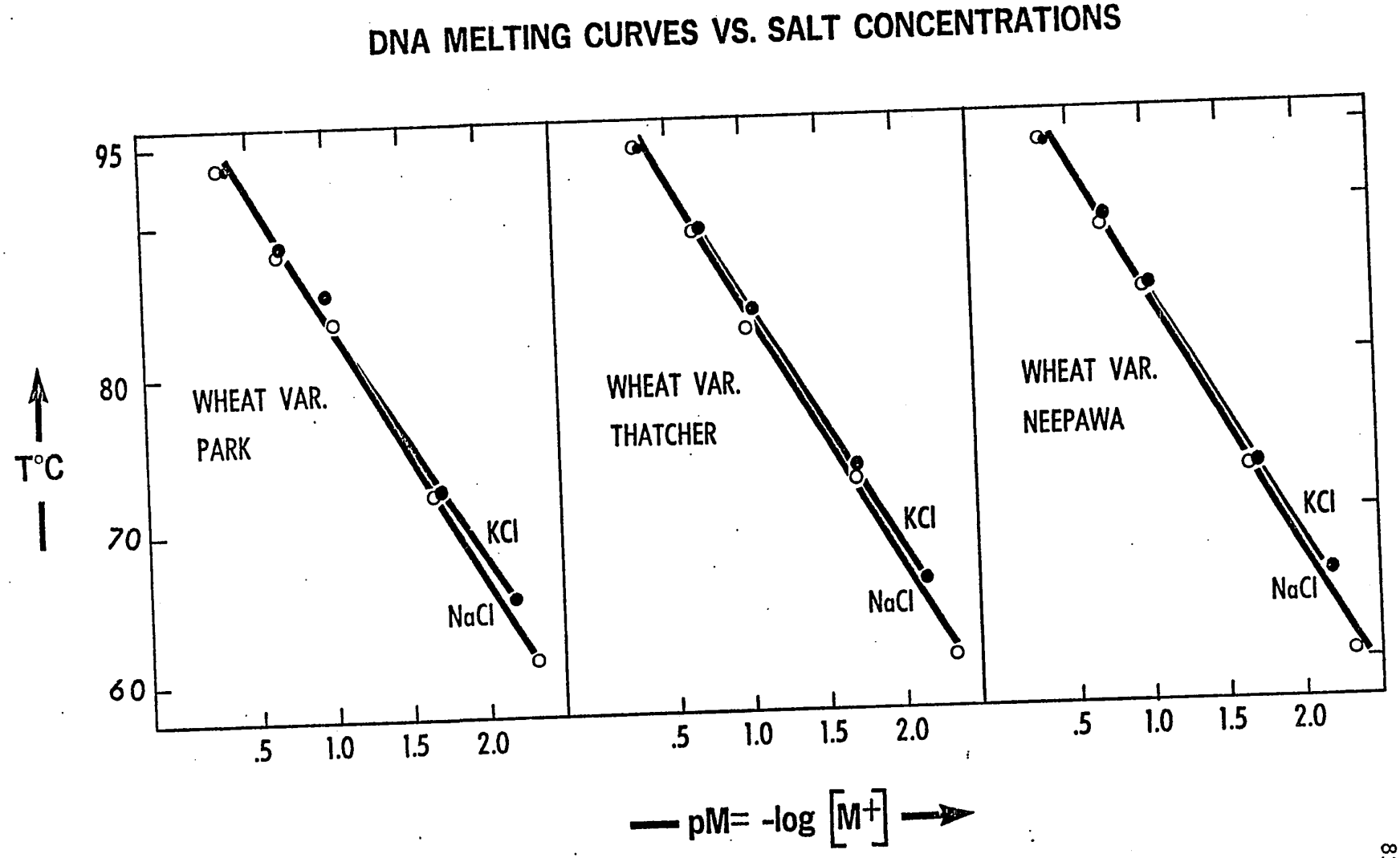


Figure 14

Melting Temperatures of Park, Thatcher, and Neepawa DNA in Various Concentrations of Sodium Chloride and Potassium Chloride

V. DISCUSSION

A. PROTEINS

The extraction procedure used for protein fractionation in this study separated flour proteins into four broad solubility groups. Proteins soluble in water constituted the albumins; while the salt-soluble proteins constituted the globulins. Gliadins extracted by 70% ethanol were obtained in the highest purity and yield. The acetic acid-soluble proteins comprised many of the gluten proteins. For yield calculations of each protein fraction, the nitrogen-to-protein conversion factor of 5.7 was used, based on a classical study of wheat proteins by Osborne (1907). From amino acid composition data of flours, that factor should be closer to 5.6 than 5.7. Nevertheless the value of 5.7 was applied since it is more reliable for proteins of whole wheat flour (Tkachuk, 1966).

1. Electrophoresis in the Presence of 3 M Urea

Our gel electrophoresis studies in the presence of 3 M urea showed that protein fractions derived from reduction rolls had more complex electropherograms than those collected from the breaking rolls. For water- and acid-soluble protein fractions, many of the differences observed were of minor importance. More distinctive differences were observed for the salt-soluble fraction in the regions of low and intermediated electrophoretic mobility. However, in all cases, there was too much overlap among varieties to be able to use the electropherogram

as a means of varietal identification.

The ethanol-soluble proteins, however, contained the most striking differences among the varieties investigated. The differences were mainly restricted to the region of low electrophoretic mobility.

According to the literature, the importance of varietal differences is controversial. Some authors report them to be of significance and others indicate that they are insignificant. Applying the electrophoretic technique similar to ours, Silano et al. (1969) presented evidence that albumins and globulins of the varieties of Triticum aestivum and Triticum durum are very different. However, within the latter, there were groups comprising more than 20 varieties, which revealed the same electropherograms for the albumins. For Triticum aestivum, the electrophoretic patterns of globulins were the same for groups comprising about 10 different varieties.

Applying starch gel electrophoresis and 0.5 M urea as a deaggregating agent, Coulson and Sim (1964 and 1965) observed varietal differences to be restricted to the region of low electrophoretic mobility. However, no correlation was established between these differences and rheological characteristics of the flours.

Doekes (1968), for water extracts of several varieties, observed differences for gliadins in the region of low electrophoretic mobility. From these differences he formed five groups which were further subclassified by differences in bands obtained in the regions of high and intermediate mobility.

Tanaka and Bushuk (1972), applying cationic gels, and instead of urea, 2 M dimethyl formamide; found distinct differences among

varieties in the ethanol-soluble fraction. In addition, some differences were revealed among albumins and globulins, but these differences were not clearly evident among the varieties.

Our results showed that among the varieties the distinctive differences were in the region of low electrophoretic mobility and that they were pronounced for the ethanol-soluble proteins. Furthermore, our results suggested that the protein content of the flour collected from the breaking and reduction rolls did not affect the electropherograms of any protein fraction studied. However, the flour from the breaking rolls with higher amounts of protein usually revealed fewer bands than that from the reduction rolls with less total protein; but with more bands. That the protein content of flour does not affect the electropherograms, was also found by Lee and Ronalds (1967) and recently also confirmed by Tanaka and Bushuk (1972).

2. Electrophoresis in the Presence of Sodium Dodecyl Sulfate

Applying electrophoresis in the presence of sodium dodecyl sulfate (SDS), a method developed by Shapiro et al. (1967), electropherograms obtained permitted the estimation of molecular weights of individual wheat protein bands with an accuracy of ± 10%. For flour collected from the breaking rolls, considering the major intensity bands only, the molecular weights found for water-soluble proteins were 65, 33, 31, and 18.5 ($x\ 10^3$); for the salt-soluble proteins: 67, 64, 59, 42, and 23 ($x\ 10^3$); and for the acid-soluble proteins: greater than 67×10^3. Between varieties the differences noted were not greater than 10%.

For proteins from the reduction rolls, the pattern of molecular weight distribution was simpler. The water-soluble fraction had only one major component of 59 x 10^3; salt-soluble of 54, 62, and 58 (x 10^3); and acid-soluble, as previously, greater than 67 x 10^3. In contrast to the above findings, distinctive differences were present among varieties in salt-soluble proteins.

For the major component of albumins, Fish and Abbott (1969) reported a molecular weight of 19.3 x 10^3 when gel filtration on Sephadex G-75 was applied; 16 x 10^3, when sedimentation equilibrium analysis was used; and 16.3 x 10^3, when amino acid analysis was applied.

Merideth and Wren (1966) using Sephadex G-200 for wheat proteins, obtained four peaks in the sequence: glutenins, gliadins, albumins, and non-proteins. For the average molecular weight of the albumin fraction, they obtained the value of 16,000.

Ewart (1969),from Cappelle-Desprez flour, isolated the albumins and obtained a molecular weight of 26,000 for the major component, using sedimentation runs and amino acid analysis. In addition, no enzymatic activity was found for this protein.

Feillet and Nimmo (1970), from common wheat flour, isolated and purified two albumins for which they reported molecular weights of 24.8, 20.1, and 19.8 (x 10^3) for the first and 13.4, 12.9, and 11.2 (x 10^3) for the second, depending whether gel permeation chromatography, amino acid analysis or sedimentation runs were applied.

Cantagalli et al. (1971) isolated and purified three albumin fractions from Triticum aestivum by gel filtration and preparative disc electrophoresis on polyacrylamide gel. Subsequent sedimentation

velocity studies, as well as amino acid analysis gave molecular weights of 17.7, 18.2, and 18.9 (x 10^3).

Though the results above were obtained by different methods of molecular weight estimation there is a similarity to results obtained by gel electrophoresis in the presence of SDS. All varieties in our studies contained a band in the region of 24,000 as well as a band at 17,800. In our system, proteins of molecular weights close to one another such as those of 24,800 and 26,000, would probably not be resolved into 2 bands. A good proportion of the albumins are located in the high electrophoretic mobility region with molecular weights below 17,800 i.e. in the region beyond the calibration points established by this method.

Some data is available for molecular weights of isolated and purified salt-soluble proteins from wheat flour. Kelly (1964) reported a molecular weight of 75,000 while a more recent study by Meredeth and Wren (1966) using an elution technique on Sephadex G-200, indicated a value of 69,000. Our results showed a triple band around 63,000, but in addition, we had bands with 42,000, 23,000, and below 18,000.

The major bands of the ethanol-soluble fraction (gliadins) as determined by the method used in this study were represented by molecular weights of 65, 45, 35.5, and 32 (x 10^3). The range of bands obtained in our study would correspond to a molecular weight range of 25,000 to 100,000 for gliadins found by gel filtration on Sephadex G-200 (Meredith and Wren, 1966). Bernardin (1967) for alpha-gliadin, obtained a molecular weight of 50,000; while beta-gliadin was reported

to have a molecular weight of 31,000 (Jones et al., 1961). Results in our studies revealed that the proteins from the reduction rolls contain a medium intensity band corresponding to a molecular weight of 53,000; while for the flour from the breaking rolls, a major band in Thatcher and Neepawa and a medium band in Park was represented by a molecular weight of 49,000.

Huebner et al. (1967) purified $gamma_1$-, $gamma_2$-, and $gamma_3$-gliadins and found their molecular weights to be in the range of 16 to 18 x 10^3. These results were calculated from amino acid analyses. However, sedimentation studies on gamma-gliadins indicated values of 26,000 (Woychik and Huebner, 1963) and 31,000 (Jones et al., 1965). Our studies revealed a major band for Thatcher and Neepawa and a medium band in Park corresponding to a molecular weight of 30.5 x 10^3 for the breaking rolls; also present in flour from the reduction rolls, but only as faint intensity bands.

The bands, approximately 10, obtained for the gliadin fractions by electrophoresis in the presence of SDS probably still do not represent the complexity of these proteins. Wrigley (1970) fractionated gliadin into a two-dimensional map of over 40 components by isoelectric focusing in the first followed by starch gel electrophoresis in the second dimension. Although our procedure resolved the gliadins into only 10 bands, varietal differences could still be observed, not only through the mobility or molecular weight, but from the quantities of protein present in the individual bands. In this respect, Park differed from Thatcher and Neepawa which were very similar to one another,

probably also reflecting their genetical similarity.

3. Reliability of Molecular Weight Determination by Electrophoresis in the Presence of SDS

As found by Weber and Osborn (1969), the accuracy in molecular weight determinations of proteins using gel electrophoresis in the presence of SDS, was better than ± 10% when polypeptide chains were analyzed in the range of 15,000 to 100,000 molecular weight. In addition, they established that the mobility in the presence of SDS is independent of isoelectric point, amino acid composition, and conformation of the protein (helical, globular, or rod-shaped in the native state) and dependent solely on the molecular weight of the protein. Formation of interchain disulfide bands was prevented by the presence of 2-mercaptoethanol in the protein samples analyzed.

As found by Reynolds and Tanford, (1970), under conditions of electrophoresis similar to those used in the present study, all protein particles bound equal amounts of SDS on a weight basis, which brought about a loss of protein specificity and the electrophoretic mobility became the measure of molecular size alone.

That the sulfhydryl-disulfide interchange and reduction of disulfide groups was relevent for flour proteins, had already been demonstrated by gel filtration on Sephadex G-200 (Meredith and Wren, 1966). Thus, by the addition of 2-mercaptoethanol to a total wheat protein extract, Meredith and Wren abolished the glutenin peak and observed the shift of molecular weight distribution to lower values. Since wheat proteins do not consist of several polypeptide chains linked by peptide bonds, but only of chains linked by disulfide bridges,

the molecular weights found in the present study could be considered reliable.

4. The Extraction Procedure Used

The extraction procedure used in the present study was essentially based on Osborne's (1907) original procedure of fractionation. It appeared to be fast, simple, and to provide high yields and has been used by many authors (Kaminski, 1962; Kaminski and Bushuk, 1969; Coulson and Sim, 1964 and 1965).

However, aside from these advantages the above method also has its drawbacks since some gliadins are extracted with the albumins as the ionic strength of the resulting flour extract decreases (Hoseney and Finney, 1971). Recently such a gliadin contamination appeared to be avoided when the initial water extraction was omitted and the flour was extracted first with 0.5 M sodium chloride followed by dialysis of the extract against water, resulting in globulin precipitation and the albumins being left in the supernatant (Chen and Bushuk, 1970).

Nevertheless, both methods provided complex protein fractions as evidenced by the present study and that reported by Tanaka and Bushuk (1972).

B. NUCLEIC ACIDS

1. Total Nucleic Acids

Our preliminary attempts to separate the wheat embryo DNA by the use of a short MAK column and a stepwise gradient according to Sueoka and Cheng (1962) did not provide a distinctive peak for DNA. Usually the small peak was screened by other ultraviolet absorbing

components, probably rRNA species, present in the embryos in large quantities. When a continuous, linear salt gradient was applied, the peak was well separated, although the yield per total A_{260} units recovered was very low. Hence, the green leaves of 4 - 5 day old shoots were used as the DNA yield from this source was comparatively larger.

The DNA peaks from the embryos and green leaves were eluted at a concentration of 0.55 M NaCl which indicated a close similarity in DNA base composition and / or size of these molecules. However, Chen and Osborne (1970) observed that DNA from ungerminated wheat embryos of Triticum vulgare differed in base composition from DNA present in germinated embryos. The melting point of the DNA from the dry, ungerminated embryos was 1.5 - 2 °C higher than that from germinated embryos. In addition, the hyperchromicity of the DNA from the germinated embryos was 7% higher. The G + C contents revealed by the data of Chen and Osborne (1970) indicated a higher value for DNA from ungerminated embryos.

On the basis of observations by Chen and Osborne (1970), our results indicated that MAK column fractionation with optimal separation conditions could not reveal differences in the base composition of DNA species. This finding was opposite to that of Sueoka and Cheng (1962) who claimed that the MAK column was able to separate DNA on the basis of base composition with that having a greater G + C content being eluted at a lower salt concentration.

The highest A_{260} units recovered from MAK columns were always in the region of rRNA, but the green leaves always contained smaller

amounts of rRNA than did the ungerminated embryos. The tRNA regions recovered were always higher for ungerminated embryos and lower in green leaves when compared to the DNA contents of these sources. Since ribonuclease activity was absent in wheat embryos (Allende and Bravo, 1966) the yields of tRNA obtained should represent the in situ proportion of tRNA, which would not be the case for tRNA recovered from the green wheat leaves. In experiments where larger quantities of wheat leaves were processed, considerable enzyme activity was observed, regardless of the presence of bentonite as an inhibitor, which decreased the rRNA region with the degraded fragments enriching the tRNA region.

2. DNA

The hyperchromicity observed with heating of the DNA of all varieties during melting temperature determinations indicated that the DNA preparations were predominantly in the native configuration. For all varieties, the wheat leaf DNA G + C content was 46.4 - 46.5 mole per cent when melting temperature determinations were carried out in SSC/10. These values were close to the 45.6 ± 3% reported by Huget and Jouanin (1972) for purified nuclear DNA isolated from etiolated 4 day old wheat shoots of the French wheat variety, Etiole-de-Choisey. The G + C content was determined to be 42.8 mole per cent as a result of buoyant density gradient centrifugation in neutral cesium chloride (Huget and Jouanin, 1972). Chen and Osborne (1970) reported a G + C content of 35.6 mole per cent as obtained by melting temperature determinations and a value of 37.8 - 38.0 mole per cent was obtained by buoyant density gradient centrifugation in cesium chloride, for the DNA from germinated wheat embryos. Bendich and McCarthy

(1970a), for the DNA from hexaploid wheat shoots, reported a G + C content of 39 mole per cent from melting temperature determinations and a value of 43 mole per cent determined from buoyant density values.

Although Chen and Osborne (1970) established that distinctive differences existed for G + C contents of DNA in germinated and ungerminated wheat embryos, it is surprising that results reported so far, for ungerminated wheat embryo DNA are very close to those reported for DNA from wheat leaves. For wheat embryo DNA, Marmur and Doty (1962) reported a G + C content of 46 mole per cent by melting temperature determinations, while Schildkraut et al. (1962) and Spencer and Chargaff (1963), using chemical base determinations, reported values of 47 and 44.5 mole per cent respectively.

The similarity of the G + C content reported for DNA from wheat embryos and green leaves should be even more surprising since the embryos contain the bulk of the DNA in the nuclei, while the DNA from young leaves should be substantially enriched with chloroplast DNA. As found by Kung and Williams (1969) the value of 5.7×10^{-15} g DNA / chloroplast was much less than 5.8×10^{-11} g / nucleus. On the other hand, the reported ratio of intact nuclei to chloroplasts was 1:150 (Woodcock and Fernandez-Moran, 1969), which might indicate that chloroplasts could comprise only 1.5% of the DNA obtainable from leaves.

In the present study, the DNA melting curves were somewhat asymmetrical and appeared to be skewed toward the G + C rich end of the curve. That this slight distortion was not due to omission of the correction for volume changes of DNA solutions during heating, was supported by similar curves recorded by Huget and Jouanin (1972) who included

the necessary corrections for thermal expansion of the DNA solutions. In addition, these authors observed slight deviations from an idealized curve shape which were also observed occasionally in our samples. These polyphasic denaturation curves for purified nuclear DNA were proposed to represent heterogeneity i.e. the presence of several families of DNA in the wheat nuclear DNA preparation.

These facts were also reflected by thermal transition interval values of 9.1°C obtained by us for DNA samples in SSC/10. Huget and Jouanin observed values of 10°C in their study.

In the determination of DNA melting temperatures in different salt concentrations, it appeared that a linear relationship existed between melting temperatures of the DNA of each wheat variety and the negative logarithm of the salt concentration applied. As indicated by Gruenwedel et al. (1971), increasing salt concentrations led to a decrease in the thermal stability of DNA samples over a wide range of G + C contents. It was suggested that the water present in the narrow groove of the DNA helix, by its additional hydrogen bonds, imparted stability to the double helix in solution. The presence of high salt concentrations was proposed to have a destabilizing effect on the stability of DNA in solution.

Assuming that such an approach might reflect possible differences in G + C rich regions of the DNA of each of the three wheat varieties investigated, a study of salt concentration effects on the melting temperature of DNA was carried out. However, as experienced by us, interferences in recording the hyperchromicity changes at the temperatures required for salt concentrations greater than 0.5 M rendered

such an approach unfeasible. Nevertheless in a range of 0.005 to 0.5 M salt concentration, a linear relationship appeared to exist which was the same for all varieties, suggesting no difference in their DNA due to the presence of heterogeneity as indicated by the large thermal transition values. In addition, there was a decrease in the thermal transition interval width as the salt concentration increased. This effect was similar to that observed by Gruenwedel et al. (1971). Furthermore, small deviations observed for melting temperatures obtained in the presence of sodium and potassium chlorides might be explained by differences in hydration affinity of potassium and sodium ions.

Thus, as well as for the protein results, data for the DNA of each variety could not support a reliable method of varietal characterization of wheat, which was the aim of this study.

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