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

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ABSTRACT

Dronzek, Bernard Leo, Ph. D., The University of Manitoba, February 1970. Nature of the Endosperm Proteins of the Extracted Tetraploids Derived from three Common Wheat Varieties.

Major Professor: Dr. W. Bushuk

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

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

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

one fraction among varieties were insignificant.

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

I INTRODUCTION

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

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

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

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

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

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

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

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

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

II LITERATURE REVIEW

1. Introduction

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

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

large number of molecularly different proteins.

2. Electrophoresis Studies of Wheat Proteins

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

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

to increase the solubility of the proteins.

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

3. Water and Salt-Soluble Proteins

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

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