A monosomic inheritance study to locate genetic factors for protein quality and morphological characters in hard red winter wheat
by James Welsh

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Genetics
Montana State University

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A MONOSOMIC INHERITANCE STUDY TO LOCATE GENETIC FACTORS FOR PROTEIN QUALITY AND MORPHOLOGICAL CHARACTERS IN HARD RED WINTER WHEAT

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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Approved:

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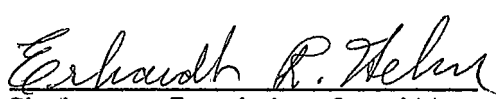

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ABSTRACT

Aneuploid lines of the hexaploid hard red winter wheat Triticum aestivum variety Kharkov MC22 were hybridized with the normal variety Itana. Genetic studies were completed on the parents and F_2 populations.

Glume color was inherited as a monohybrid character associated with chromosome 1B. Two factor pairs governing auricle color were associated with chromosomes 4A and 5B. Factors influencing date of heading were associated with chromosomes 3A, 5B, and 4D.

In the inheritance of gluten quality, chromosome 1D, in the monosomic condition, had a pronounced effect on the Kharkov parent and the segregating F_2 population. The lack of this chromosome resulted in the radical reduction of gluten strength as measured by the wheat meal time fermentation test and farinograph test. Other chromosomes associated in minor ways with gluten quality were 1A, 4A, 4B, and 4D.

INTRODUCTION

The economically important problem of wheat protein quality inheritance has perplexed the plant breeders and geneticists for many years. The fact that protein quality is extremely important in the commercial flour industry warrants investigation of this character; and if genes influencing protein quality could be localized, the investigation of gluten formation and structure would be greatly facilitated. The complexity of the genetic system combined with pronounced environmental effects makes genetic study by conventional methods extremely difficult. The purpose of this investigation was to determine the chromosomal location of genetic factors involved in protein quality inheritance, utilizing aneuploid lines of hard red winter wheat as the genetic material. It was hoped that by crossing the aneuploid lines of Kharkov MC22, which normally has a weak gluten, with the strong gluten variety Itana, and studying both the parents and F_2 populations, it would be possible to detect deviations from the normal population distributions when chromosomes carrying factors for protein quality were tested. It was suspected at the onset that the major factors should be located in the D genome, which is distinctive to the bread wheats.

In the course of the investigation the morphological characters of glume color, auricle color, and heading date were also to be located with respect to the proper chromosomes. Deviations from normal F_2 ratios were to be taken as an indication that a critical chromosome had been encountered. While the characters of glume and auricle color are not very

important commercially, their linkage group identification would allow them to serve as useful genetic markers. Although date of heading is probably governed by a complex genetic system, any specific information regarding its inheritance would be of value to the plant breeder.

REVIEW OF LITERATURE

I. Aneuploid Inheritance.

Aneuploidy refers to a condition in which the chromosomal number of the individual has been changed by some number other than the exact multiple of a basic monoploid number. Terms commonly encountered in aneuploidy are tetrasomic (2n+2), trisomic (2n+1), disomic (2n), monosomic (2n-1), and nullisomic (2n-2).

The value of aneuploid inheritance in locating genetic factors with respect to specific linkage groups has been demonstrated by various workers. Blakeslee (10) in his classic studies of the trisomics of Jimson weed, Datura stramonium, (n=12) showed that each of the 12 chromosomes, when in the triploid condition, produced a visable specific change in capsule size and shape. Blakeslee also studied the effects on the plant of other types of chromosomal aberrations such as variation in ploidy level, and combinations of aneuploids and polyploids resulting from hybridization of appropriate plants. By means of extra chromosomes and parts of chromosomes it was possible to secure a wide range of variations in Datura affecting the structure and physiology of all parts of the plant that were studied.

Clausen (16), and Clausen and Cameron (17) established a complete series of monosomic lines of tobacco Nicotiana tabacum (n=24). They established their lines primarily through the use of an asynaptic gene, pale-sterile. Through hybridization of plants carrying the

pale-sterile gene with normal plants and making appropriate back-crosses, they were able to reduce the unbalanced diploids to simple monosomics and trisomics. They outlined in detail a genetic program utilizing the monosomic individuals to determine the location of genes with respect to specific chromosomes, and then went on to demonstrate effectively their theories by locating no less than 18 different morphological factors. Essentially, they suggested the hybridization of a monosomic individual carrying dominant alleles for a character with a normal individual carrying recessive alleles for the character. If both dominant and recessive are expressed in the F_1 progeny, one can then assign that factor to the particular chromosome in the monosomic condition. They express the opinion that this type of program would not lend itself to a self-polinated species such as wheat because of the excessive work necessary to produce the F_1 seed.

The utilization of trisomics in the tomato, Lycopersicon esculentum (n=12), has been reviewed by Rick and Butler (55). As in Datura, the extra chromosomes exert such a strong developmental and physiological effect that each of the 12 different trisomics can be distinguished readily, even in mixed genetic backgrounds. It is possible, furthermore to differentiate the trisomics from the diploids in the seedling stage. The most striking and diagnostic changes were observed in plant growth habit, shape and size of all organs, and color and texture of leaves.

In Drosophila melanogaster the monosomic condition for the small chromosome IV has been observed and Bridges (11) describes these individuals as differing from normals by having pale body color, shortened wings, roughish eyes, slender bristles, reduced fertility and high mortality.

Deficiences of whole chromosomes or parts of chromosomes in 21-chromosome wheat have been observed by various workers. Kihara (36), as cited by Sears (60), obtained in the F_4 of a cross between T. polonicum (n=14) and T. spelta (n=21) two plants which had only 20 pairs of chromosomes and which bred true. One was a dwarf with a somewhat lowered seed set, while the other was semi-dwarf, with nearly normal fertility. Nishiyama (47), as cited by Sears (60), studied the monosomic plants which were obtained from these two lines through crosses with normal plants. He found that such monosomics produced functioning 20-chromosome gametes, though the proportion was much lower among male than among female germ cells.

The first extensive aneuploid genetic program in wheat was initiated by Sears (60) in 1939 when he observed two haploid plants in a field culture of common wheat, Triticum aestivum variety Chinese Spring (n=21). These haploids both showed complete male sterility and one was completely female sterile. The female fertile plant set 14 seeds as a result of pollination with normal wheat pollen. The 14 seeds yielded 13 mature plants which ranged from 40-42 in somatic chromosome numbers.

Each of these plants was investigated cytologically at meiosis. Five of the 41 chromosome plants showed 1 univalent and 20 bivalents, and 2 showed 2 univalents and 1 trivalent each. Of the two 40 chromosome individuals, 1 showed 19 bivalents and 2 univalents, and the other showed 17 bivalents, 2 univalents, and a ring of 4. Of the four 42 chromosome plants, 2 were normal with 21 bivalents each, 1 had 15 bivalents, 2 univalents, 2 trivalents, and a ring of 4, and the other had 19 bivalents, 1 trivalent and 1 univalent. Sears hypothesized that the different meiotic configurations in the progeny were the result of abnormal division or segregation of the 21 univalent chromosomes at meiosis of the original haploid plant. The resulting gametes which carried either duplications or deficiencies for one or more of the chromosomes, when combined with the normal male gametes, would result in progeny which were abnormal in their chromosome numbers and pairing configurations. He felt that the rings of four were the result of reciprocal translocations rather than a tetrasomic condition.

To determine partial chromosome identification of the monosomic individuals, Sears made hybridizations between them and the 14 chromosome emmer wheat Triticum durum. Sax (59) showed that the 14 chromosomes of emmer (durum) wheat regularly pair with 14 of the 21 T. aestivum chromosomes. If Sears' monosomic individuals were monosomic for chromosomes of the emmer group, hybridization between the monosomics and the durum should result in meiotic configurations

with eight univalents. If they were not of the emmer group, six univalents should appear. He found that of the eight different monosomic lines he had developed, five proved to have a homolog in the emmer complement, while three were among the extra seven chromosomes (D genome) of I. aestivum.

Sears (60) investigated the morphological characteristics of the progeny which resulted from selfing each of the eleven original plants with chromosome aberrations. By combining cytological studies with morphological observations, he was able to assign relatively specific phenotypic effects to the monosomic, trisomic and nullisomic conditions. Major differences involved color, plant size, spike shape and fertility. Several of the nullisomic individuals did not reproduce.

Sears (61) describes seven of the nullisomics which resulted from the original monosomic individuals. He notes that all of the seven are of reduced fertility, but none are completely sterile. Four are both male and female fertile. Some of the characters affected by the nullisomic condition include lax spikes, tillering capacity, culm length, vigor, awnedness and sterility. Here he also notes the expression of partial asynapsis in nullisomic III. This proved to be a very valuable line because of the ease with which it generated other monosomics and nullisomics in a hybridization program.

By 1944 Sears (62) had obtained 17 of the possible 21 nullisomics and monosomics, and 11 tetrasomics. Of the 17 nullisomics and

monosomics, 11 had homologs in the AB (emmer) complement and 6 were of the D genome. From observations of the nullisomic individuals, the factors governing seed color, pubescent nodes, squareheadedness and suppression of speltoidy, and awn development, were assigned to specific chromosomes. His observations on the frequency of transmission of the nullisomic and monosomic conditions from selfing monosomic plants indicates that nullisomics are produced in frequencies ranging from 3 to 10 percent, while monosomic frequency ranged from 75 to 85 percent. Elliot (23) points out that in the production of female gametes there is approximately a 50 percent chance of the univalent being lost in the first meiotic division and again a 50 percent chance of it being lost in the second division, thus resulting in the production of approximately 75 percent female gametes deficient for the chromosome. Apparently there is little selective advantage of the normal gametes over the deficient gametes in the female. The frequency of production of deficient male gametes would be approximately the same as in the female; however, the low number of functioning deficient male gametes is presumably due to considerable elimination of deficient pollen through competition with normal pollen. The differences in frequencies of nullisomic production from various selfed monosomics partially indicates the differences in competitive ability of the deficient male gametes.

Sears (62) in 1944 observed two instances where one chromosome in the tetrasomic condition canceled, in part or almost entirely, the

effects of a different chromosome in the nullisomic condition. These chromosomal combinations were obtained by making hybridizations between individuals trisomic or tetrasomic for one chromosome with individuals nullisomic for a different chromosome. He suggests that the ability for compensation is due to homoeology or identity of one or more segments between the two different chromosomes in question. The common segments were not long, however, because no pairing was observed when plants monosomic for both chromosomes involved in the compensating situations were obtained. Further evidence of duplications in T. aestivum chromatin is given by Stadler (74). He found fewer mutations following X-ray treatment of the hexaploid T. aestivum wheat than following treatment of diploid and tetraploid wheats, indicating that many gene loci are masked by duplicate genes at other loci. The fact that chromatin duplications occur in T. aestivum probably accounts for the relatively high viability of the monosomics and nullisomics in this species as compared to diploid species such as Datura stramonium.

By 1952 Sears (64) had grouped 20 of 21 chromosomes into 6 groups of 3 each and 1 group of 2 by use of the compensating tetrasomic nullisomic combinations, and termed each chromosome within a group as homoeologous or partially homologous with the other chromosomes in the group. He noted that one chromosome from each of the seven groups belonged to the D genome and hypothesized that each of the homoeologous groups consists of one chromosome each from genomes A, B, and D, the

three chromosomes from each group having been derived from the same ancestral chromosome.

All 21 of the aneuploid lines, including nullisomics, monosomics, trisomics, and tetrasomics, were reported by Sears (68) in 1954. He discussed the morphological effect each chromosome had on the plant when in a deficient or duplicated condition. He also observed that the 21 chromosomes fell into 7 homoeologous groups of 3 each, based upon the compensating tetrasomic nullisomic situations.

In 1958 Sears (69) reported the final complete grouping of all 21 chromosomes into 7 homoeologous groups and also designated the genome to which most chromosomes belonged. In regard to the latter designation, the D genome chromosomes were relatively easy to determine by crossing the monosomics of the hexaploid (ABD) with a normal tetraploid (AB) and observing the number of univalents. The A and B genome designations were partially determined by Okamoto (49) when he utilized genomically unidentified telocentric monosomics in a hybridization program with a previously produced AADD amphidiploid. If heteromorphic bivalents were formed, the chromosome was assigned to the A genome and if no bivalent association was formed, it was assigned to the B genome. These findings led Sears to suggest a new system to number the hexaploid wheat chromosomes based on their genome location and homoeology. In the previously used system the chromosomes were numbered with Roman numerals I-XXI. Under the new system the chromosomes are designated with a combination of Arabic numerals,

1 to 7, and a capital letter, A, B, or D, depending on the genome in which they are located (Table I.).

Table I. New numbering system for chromosomes of hexaploid wheat.

Homoeologous Group	Genome A		Genome B		Genome D	
	New	Old	New	Old	New	Old
1	1A	XIV	1B	I	1D	XVII
2	2A	XIII	2B	II	2D	XX
3	3A	XII	3B	III	3D	XVI
4	4A	IV	4B	VIII	4D	XV
5	5A	IX	5B	V	5D	XVIII
6	6A	VI	6B	X	6D	XIX
7	7A	XI	7B	VII	7D	XXI

The new numbering system will be used throughout this paper.

Upcott (84) and Darlington (22) have shown that univalent chromosomes, through misdivision of the centromere, may give rise to telocentric chromosomes, which are completely deficient for one arm, or to isochromosomes, which are deficient for one arm and duplicated for the other arm. Sears (62) in 1944 observed aberrations of both these types in the T. aestivum monosomic material. At this time he had obtained either an isochromosome or telocentric or both for one or both arms of the 15 of the 17 identified monosomics. These individuals occurred with a relatively higher frequency than the nullisomic individuals, indicating a selective advantage of the male gametes carrying the isochromosome or telocentric over the completely deficient gamete. Sears (65,66) gives a complete summary on the frequency of isochromosomes and telocentrics resulting from centromere misdivisions, and the behavior of these aberrations at mitotic and

meiotic divisions.

In 1948 Sears and Rodenhiser (70) reported the identification of a chromosome bearing factors for the economically important character of wheat stem rust resistance. This was accomplished by hybridization of the rust resistant variety Timstein with the monosomic lines of the susceptible Chinese Spring variety and studying F_2 ratios. All but one F_2 population agreed with the expected ratio of approximately 9 resistant : 7 susceptible. The deviating F_2 family which involved nullisomic chromosome 6B showed 2 susceptible plants and 122 resistant out of the 124 available for testing. Weak linkage of these two dominant genes had previously been postulated and the nullisomic analysis proved conclusively that this was the case. This evidence demonstrated the usefulness of the aneuploid series in determining the chromosomal genetic location of factors in hexaploid wheat, and also promised answers to some of the difficulties involved in establishing linkage groups in this organism.

Following this, the wheat aneuploids were utilized by a number of workers in wheat genetics. Unrau (82) located factors for glume color, winter habit, awning, bunt resistance modification, and spike density. His program involved hybridization of monosomic or nullisomic Chinese Spring lines with normal varieties and studying F_2 and F_3 populations for segregation ratios. Heyne and Livers (33) located factors for leaf rust reaction, awnedness, winter injury, and seed color in Pawnee wheat utilizing the F_2 analysis method. Nyquist (48)

identified a chromosome carrying factor for stem rust resistance. An extensive investigation has been conducted by Larson (39) and Larson and MacDonald (40, 41, 42) regarding genetic factors influencing stem solidness in winter wheat.

Morris (43, 44, 45) has compiled complete lists with additions and revisions of chromosomal locations of genes for hexaploid wheat characters.

Sears (67) outlined the possible methods for utilizing aneuploids in genetic analyses of wheat. These methods included: (1) ascertaining that the effect of a particular gene is absent in a nullisomic; (2) growing F_2 populations from crosses with each of the 21 nullisomics or monosomics and determining which F_2 family segregates abnormally; (3) analyzing selected F_2 plants in the F_3 generation; and (4) substituting the individual chromosomes of other varieties into a standard variety. In the methods involving the analysis of F_2 and F_3 populations it becomes apparent that if monosomic F_1 plants are selected, the source of only one chromosome is positively known, that is the F_1 monosomic chromosome must have come from the disomic parent, while the other chromosomes in the complement are segregating at random. In opposition to this, the substitution program allows for the determination of the effect of one chromosome pair in a specifically known chromosome complement background. The chromosome substitution program involves the crossing of a particular monosomic or nullisomic individual to a normal individual of the variety from

which the chromosome is to be substituted. By repeated backcrossing using the monosomic as the recurring female parent and selecting monosomic individuals each generation, it is possible to regain the genetic constitution of the monosomic individual, with the exception of the chromosome contributed by the original normal parent. The production of different monosomic varieties can be accomplished in much the same manner, except that the original normal parent is used as the recurrent parent in the backcrossing program. The advantages of using nullisomics as compared to monosomics in a substitution program are discussed by Unrau (83). If the nullisomic is used, the identity of the univalent is always known to be that of the desired variety. However, when the monosomic is used there is a male transmission of the deficiency ranging in frequency from 1 to 10 percent of the fertilizations, according to Sears (62). Should this occur in combination with a normal female gamete, the resulting offspring would be monosomic for the chromosome of the original monosomic parent rather than for the desired chromosome of the normal parent. This situation can be overcome by selfing the hybrid between each generation and using only the recovered disomics as the female parent; however, this involves twice as many generations as the program involving nullisomics.

An example of the utilization of substitution lines in genetic analysis of wheat is provided by Kuspira and Unrau (38). They were able to study the chromosomal association of genes governing awning,

earliness, lodging, plant height, spike density, 1000-kernel weight and yield in this manner.

II. Glume Color Inheritance

Color of glumes on mature T. aestivum is expressed as a red or bronze, while lack of color is designated as white. The inheritance of red or bronze glumes as a single factor pair has been reported by Biffen (8), Clark and Hooker (14), Clark, Quisenberry and Powers (15), Florell (27), Kezer and Boyac (35), Stewart and Bischoff (77), Stewart and Nelson (78) and others. Both monogenic and digenic inheritance has been reported by Torrie (80). Unrau (82) determined, from aneuploid studies, that the dominant gene for red color in the variety Federation 41 was associated with chromosome 1B. Sears (68) noted that the nullisomic for chromosome 1B in Chinese Spring displayed darker glumes than the normal.

III. Auricle Color Inheritance

The character of auricle color is displayed as a purple or red pigmentation in the auricle of the flag leaf. This coloration is contrasted to the lack of purple color resulting in a pale green or nearly colorless auricle. Kjanus (37) as cited by Sears (63) reports a single, dominant gene for red auricle over colorless. Villanueva-Nova (85) studied the segration of progeny resulting from a cross between red and green auricle parents. The results showed 219 individuals with red auricles and 76 with green auricles indicating a single factor pair with dominance. These results were confirmed by

studies of F_3 families. To date, no data are available from aneuploid studies which would locate this factor on any particular chromosome.

IV. Heading Date Inheritance

Of some concern to the wheat breeder is the length of time required for a variety to reach maturity. In general, the major criterion used to judge maturity time is date of heading. It would seem logical, as considerable research has indicated, that this character may be the final expression of a series of genes affecting a number of physiological processes of the plant. It is important, also, to acknowledge the fact that this character may be critically affected by environmental conditions. Biffen (8) suggests one factor pair governing earliness in wheat. Florell (26) reports that this character is governed by one major factor pair showing dominance toward earliness, with the possibility of a number of minor modifying factor pairs. Harrington (30), Stephens (76), and Florell (27) all suggest two or more factor pairs with dominance or partial dominance for earliness. Freeman (28) and Bryan and Pressley (12) found dominance or partial dominance for lateness. Nandpuri and Foote (46) report earliness of heading as partially dominant and controlled by at least two factors. Crumpacker and Allard (19) using a ten-variety diallele system to analyze heading date suggested that three independent gene pairs expressing partial cumulative dominance for earliness were controlling this character. The complexity of earliness inheritance is indicated by Kuspira and Unrau (38). Utilizing three

different substitution lines they were able to demonstrate that all but four chromosomes had an effect on date of heading.

V. Protein Quality Inheritance

The character of protein quality is strongly influenced by environmental effects, making the determination of its inheritance very difficult. In addition, the problem has been compounded by the lack of a specific critical test that can be utilized in early segregating generations when the amount of material available for testing is small. The proof that gluten characteristics are inherited is not lacking, but the determination of the number of genetic factors involved is still subject to considerable investigation. Biffen (9) utilized several crude tests in an effort to determine strength in wheat. He was unable to show a specific mode of inheritance, but did determine that this character was inherited and felt that it was governed by a relatively few factor pairs. Saunders (57), on the other hand, felt very strongly that the inheritance of strength is governed by a complex mendelian system. Saunders also points out that the physical appearance of the wheat itself is not generally a good criterion for decisions regarding the strength of the wheat. Clark, Florell, and Hooker (13) attempted a study of quality inheritance using crude protein content and kernel texture of the wheat as the basis of their evaluation. They proposed that several genetic factors were governing these characters. Others to suggest multiple gene inheritance were Ausemus, Markley, Bailey and Hayes (1),

Cutler and Worzella (21), Hayes, Immer, and Bailey (32), and Worzella and Cutler (90). Worzella (88), utilizing the wheat meal fermentation test on F_2 and F_3 material, suggested three factor pairs with additive effect. He also recognized the fact that the direction in which the cross was made had an effect on the doughball time of F_1 seed. This is probably due to the 3n condition of the endosperm in which two maternal and one paternal homologous chromosomes are present. Worzella (89) suggested that one, three, and four factors, respectively, for the three varieties investigated were governing gluten strength in a series of three-way crosses. Again the wheat meal fermentation test was used. Beard and Poehlman (5) utilizing pearling indices as an indication of quality differences between hard and soft wheats concluded that the distribution of segregates indicated a multigenic mode of inheritance. Gfeller and Whiteside (29) studied inheritance of quality as measured by expansion, sedimentation, and mixograph tests in relationship to agronomic characteristics in hard red spring wheat. Although they were able to determine some linkage between quality characteristics and rust resistance and compact spikes, the particular mode of inheritance for quality was not determined.

It is obvious that considerable confusion arises in comparison of data which comes from different analytical procedures. In the case of quality inheritance, the methods have varied from chewing the kernels to running complete baking tests on advanced generation material. If

tests are run on very early generations, the amount of grain available is usually too small to lend itself to accurate standard testing procedures. If later generation material is used for complete milling and baking tests, the plant breeder is faced with the problem of propegating a large number of lines to insure the complete testing of all genotypes. These facts, combined with year to year variation due to environment, have presented serious problems in accurately determining protein quality inheritance. It seems likely that the genetic systems proposed in the early work were governing the reaction to the test utilized. Each test probably represented only part of the quality picture. The difficulty of identifying this genetic system is emphasized by the lack of reports in recent literature.

Although wheat protein, as such, is not the major point of study in this paper, it may be well to give this subject a brief review as it is so closely associated with some genetic aspects of the experiment.

Wheat kernels may vary in total protein content from a low of around 9% to a high of 17% or more. Within the total protein there are several fractions, the most important of which, from the bread maker's standpoint, is the acid soluble portion, gluten. It is primarily this portion that helps account for such properties as elasticity of the dough, water absorption, gas production and retention, and diastatic powers. A considerable amount of research has been done in an effort to determine the specific differences between good and

poor quality gluten. However, many difficulties have been encountered in the separation of the various components. With the advent of satisfactory electrophoretic methods more accurate separations and results could be obtained. Coats and Simmonds (18) investigated moving boundry electrophoresis and DEAE-cellulose ion exchange resins for separating the components of flour proteins. They report that the DEAE-cellulose gave superior results regarding clearness of separation. They report less protein denaturation by this method than by many previously used. Simmonds (72) utilizing DEAE-cellulose was able to demonstrate eight subfractions of the pyrophosphate-soluble fraction of the wheat protein. In addition, Sangers' method (56) was utilized to determine the N-terminal amino acids in some of the subfractions. Although this is not the fraction most interesting from the flour quality standpoint, it is an excellent demonstration of methods now available for wheat protein studies. Bell and Simmonds (6) re-emphasized the importance of gluten in flour characteristics. They report a high positive correlation between the formic acid soluble (gluten proteins) nitrogen and baking score. They found no significant correlation between the amount of pyrophosphate-soluble nitrogen (albumins, globulins, pentosan-protein complexes, nucleoproteins) and baking score. They feel their findings quite logical in view of the different roles played by the pyrophosphate-soluble proteins as compared with the formic acid-soluble group. The former will contain those proteins and enzymes concerned

directly or indirectly with cellular metabolism, either in gluten or starch synthesis during grain maturation, or with their subsequent breakdown during germination. There is no evidence that any proteins of the pyrophosphate-soluble group are involved in the formation of the cohesive network characteristic of the formic acid-soluble group when flour-water doughs are prepared. Pence and Mecham (52) report the fractionation of wheat proteins through ion exchange chromotography on cellulose derivatives, electrophoresis particularly in aluminum lactate buffer, and starch-gel electrophoresis. They reported at least 9 components in gluten protein. Present in non-gluten protein are at least 3 globulins and 11 or more water soluble fractions.

Proteolytic enzyme systems are of considerable importance in the consideration of the gluten problem. According to Harris (31) too high a proteolytic activity in the fermenting dough results in weak, runny dough which is incapable of retaining the gas formed during fermentation and produces flat badly-shaped loaves. On the other hand, doughs which have harsh "bucky" glutens may be improved by the addition of appropriate quantities of proteolytic enzymes. Apparently these proteolytic enzymes operate in the presence of an activator. Glutathione has been investigated in the role of an activator by Jorgenson (34). He concluded that this compound could, indeed, act as an activator and could, if present in sufficient quantities, result in the ruin of the gluten during the fermentation process. Balls and Hale (2) concluded that flour proteases could be

activated by glutatione or by cysteine. Flobel (25) studied the action of various oxidizing agents on flour and concluded that oxidation resulted in reduced proteolytic activity, probably through oxidation of the activator.

VI. Wheat Meal Fermentation Time Test

The wheat meal fermentation test, devised by Saunders (58), was originated to fill the need for a method of analysis of very small samples with relative rapidity, while maintaining a reasonably good correlation with the standard tests used as criteria in evaluating flour characteristics of larger sized flour samples. Essentially the test involves the addition of a yeast solution to wheat meal or flour. The resulting mass is kneaded into a ball of medium consistency and placed in a container of water maintained at a constant temperature of approximately 86° F. The ball immediately sinks to the bottom, then rises as the yeast begins production of CO_2 resulting from fermentation of available sugar. As the fermentation process continues, the doughball expands and after a period of time begins to disintegrate allowing pieces of dough to fall to the bottom of the container. Time is usually measured as the period between immersion and disintegration of the doughball. Saunder's original method utilized refined flour as the basic material. However, Cutler and Worzella (20) and Pelshenke (51) initiated the modification of using wheat meal instead of flour. It was felt that the meal gave quicker, more clear-cut and dependable results than when the refined flour was used. The

passage of the meal through a 1 mm mesh sieve provides a product of satisfactory texture for the test.

The criterion of judgment is that the wheats with stronger flour characteristics have longer times, while the weak wheats have shorter times. Pool and Patterson (54) in studies on the fermentation time test have proposed an explanation for the cause of doughball collapse. They suggest that the amount of available sugar is responsible for the CO_2 production. When the sugar is fermented by the yeast, CO_2 forms small gas cells in the dough. Gradually the cells are widened and stretched out. Proteolytic enzymes working in the films surrounding the gas cells weaken the elasticity of the gluten while the pressure of the CO_2 keeps open the pores and channels in the doughball allowing a high degree of hydration in the starch and gluten. This brings about the weakening of gel films and a final collapse of the doughball under gravitational forces. It would seem, however, that the physical structure of the proteins, especially regarding elasticity properties, would play an important role in the rapidity of disintegration. It seems logical that both protein properties and enzymatic activities are measured by this test. It is also possible that both of these factors play a major part in the evaluation of wheat flours by commercial methods.

The process has been modified a number of times regarding the amounts of ingredients that are used, as well as the size of container and volume of water. Cutler and Worzella (20) suggested using 10 g.

of wheat meal, 5.5 cc. of 10 percent yeast solution, and 80 cc. of distilled water in a 150 cc. beaker. Pelshenke (51) suggested using 5 g. of wheat meal, 0.25 g. of yeast and a "quantity of water" for making the doughball, and using a beaker 6 cm. in diameter and 7 cm. high with 75 cc. of water. Bayfield (3,4) attributed a considerable amount of the variation experienced by previous workers in this test to the size of the beakers used in comparison to the amount of wheat meal used. He suggested that the wheats with longer times had a tendency to spread out on the surface of the water and receive support from the sides of the beaker if the doughball contained an excessive amount of material originally. This support tended to increase time by not allowing the proper disintegration of the doughball. He suggested reducing proportionately the amounts of meal and yeast until the fully expanded doughball did not receive any support from the sides of the beaker.

Pelshenke (51) reported that the doughball data agreed quite closely with farinograph and loaf volume data, although no correlations were run. Cutler and Worzella (20) in 1931 report no correlation between time and loaf volume, but good correlation between time and absorption, and fair correlation between time and flour protein. However, four years of quality data were correlated with time by Cutler and Worzella (21) in 1933. The ranges of correlations for the four years of the following characteristics with time were: flour protein +.49 - +.60, loaf volume + .07 - +.84, and water absorption

+.54 - +.85. Winter and Gustafson (87) gave a time correlation over two years with wheat protein as 0.30, with loaf volume as 0.72, and with dough expansion as 0.66. Wilson, Markley and Bailey (86) in testing both winter and spring varieties reported no correlation between time and protein in either case, but found a significant positive correlation with spring wheat time and loaf volume and a non-significant positive correlation of winter wheat time and loaf volume. The entire strength score, which included loaf volume, texture, and grain, of both winter and spring varieties showed a significant positive correlation with time. Kolar and Hehn[1/] report highly significant correlations between time and the flour characteristics of loaf volume and mixing tolerance, but little correlation between time and flour protein content. Allen and Hehn[2/] report significant positive correlations between doughball time and both loaf volume and mixing time. Assuming that the doughball test is a reasonably accurate method of measurement of wheat flour properties attributed to protein, it seems that investigations utilizing this test indicate

1/ Kolar, J.J., and Hehn, E.R. The use of the wheat meal fermentation time test in wheat improvement programs as a means of evaluating milling and baking quality of hard red winter wheats. Unpub. senior thesis, Plant and Soil Sci. Dept., Mont. Sta. Coll. 1950.

2/ Allen, C.D. and Hehn, E.R. The use of wheat flour in the fermentation time test to evaluate some hard red winter wheats and the protein and amino acid content of wheat. Unpub. senior thesis, Plant and Soil Sci. Dept., Mont. Sta. Coll. 1953.

that protein quality rather than quantity is of major importance in acceptable flours. Laubach and Hehn[3/] investigated further modifications of the doughball test in an effort to achieve a greater degree of accuracy in the results. The modifications included changing the yeast solution from 10 percent to 37.5 percent, and adding both glucose and potassium bromate. The 37.5 percent yeast solution was utilized mainly in an attempt to uniformly reduce time of disintegration by increasing the amount of CO_2 produced. It was found that any further increase in concentration had no reducing effect on time. The glucose was added in a 5 percent solution to insure a sufficient supply of available sugar for yeast growth, thus reducing the possibility of insufficient gas production because of lack of available sugar. The potassium bromate in a 0.2 percent solution was added to improve the quality of the meal through oxidation of the protease activator glutathione. The modified method utilized 10 g. of wheat meal, 5 ml. of the 37.5 percent yeast solution, and 1 ml. each of the glucose and potassium bromate solutions. The normal method involved 10 g. of wheat meal and 5 ml. of 10 percent yeast solution. The modified test showed a highly significant positive correlation of

3/ Laubach, V.F., and Hehn, E.R. The use of the wheat meal fermentation time test in wheat improvement programs as a means of evaluating milling and baking quality of hard red winter wheats. Unpub. senior thesis, Plant and Soil Sci. Dept., Mont. Sta. Coll. 1953.

0.60 with loaf volume, while the normal method showed a significant positive correlation of 0.46. The modified test enabled the doughball to break faster and produce a more definite end point than the normal procedure.

MATERIALS AND METHODS

Twenty-one monosomic lines, each monosomic for a different chromosome, of the hard red winter wheat Triticum aestivum variety Kharkov MC22 (hereafter referred to as KMC22) were obtained as seed in the fall of 1960 from Dr. B. Charles Jenkins, Department of Plant Science, University of Manitoba, Winnipeg, Canada. These lines had been produced by a backcrossing program with the monosomic Chinese Spring variety. This program utilized initial crosses of monosomic Chinese Spring with normal KMC22, followed by the selection of monosomic F_1 plants. Subsequent backcrosses were accomplished by using normal KMC22 as the recurrent male parent and the monosomic progeny from the backcrosses as the female. The backcrosses had been completed for eight or nine generations to insure relatively pure genetic recovery of the Kharkov complement. One exception to this was the line monosomic for chromosome 6D which had a history of only three backcrosses. KMC22 is classified as a white glumed, red auricled, late maturing wheat with poor dough quality characteristics. The normal parent chosen for this study was the variety Itana which is characterized as having red glumes, colorless auricles, early maturity, and relatively strong dough characteristics.

Ten seeds each of monosomic KMC22 lines, 50 seeds of Itana, and 10 seeds of normal KMC22 were seeded in two inch peat pots in the greenhouse on October 1, 1960. When the plants reached approximately the two leaf stage they were transferred to a cold chamber for vernalization. During the vernalization period of six weeks the chamber was operated on a 24 hour

cycle of 8 hours at 55°F with all lights on and 16 hours at 35°F with all lights off. This schedule was devised to simulate as closely as possible field conditions in the fall, and to encourage tillering. Following vernalization the plants, including pots, were transplanted to wooden soil filled benches in the greenhouse. All greenhouse soil utilized in this experiment was steam sterilized prior to use. Upon heading, hybridization was accomplished between the KMC22 monosomic lines and Itana, and also between the normal KMC22 and Itana. In all cases Itana was utilized as the male parent. The goal of six crosses on each monosomic line was established to ensure at least one monosomic F_1 line for each chromosome. It was known that each monosomic line originated from a monosomic plant, but because of the approximate ratio of 75 percent monosomic to 25 percent disomic progeny resulting from a self pollinated monosomic individual, the chromosomal number of each of these individuals could not be known definitely until cytological examination had been completed. The goal of six crosses, however, was not realized in many cases because the early maturity of Itana and the late maturity of KMC22 presented the problem of matching viable pollen with proper maturity of the females. Also, the desired amount of tillering, especially in the monosomics, was not achieved.

Head samples for pollen mother cell cytological analysis were taken on plants of monosomic lines about three days prior to emergence from the boot. The heads were preserved in Carnoy's fixative of 1 part glacial acetic acid: 3 parts chloroform: 6 parts absolute alcohol. These were then

stored under refrigeration at about 1°C until the cytological examination could be completed. Slide preparations were made according to Belling's method (7) by placing the anthers in a drop of 5% acetocarmine and squashing with a pair of dissecting needles. No heat was applied because preliminary observations indicated adequate chromosomal staining and less breakage of the cells without heat application. Following squashing, the debris was removed with forceps and a coverslip applied. Thumb pressure was applied to remove excess stain from beneath the coverslip and also to increase the desired one plane position of the cells. If slides were to be preserved for any length of time, the edges of the coverslip were sealed with clear fingernail polish. Late prophase I or metaphase I was preferred because of ease of observing univalents in these stages. If these stages were not available, telophase I or telophase II was accepted in which the occurrence of micronuclei was taken as a criterion of a monosomic condition. Chromosome counts were made when possible, otherwise the occurrence of a univalent in the cell was taken as an indication of a monosomic condition. A slight change in the staining procedure was suggested by Larson[4/]. The change involved doing a rapid squash manipulation of the anthers in the acetocarmine and immediately observing the preparation under the 10X objective without the use of the coverslip. In this way it was possible immediately to determine if the proper stages were present. If they were present, preparation of the slide was completed

4/ Larson, Ruby I. Canadian Agricultural Research Station, Lethbridge, Alberta. Personal communication. 1961.

as previously outlined. If they were not present, the slide could be wiped clean with cheesecloth and a new set of anthers prepared. This method contributed a considerable saving in time as it was necessary to prepare completely only those slides that contained pollen mother cells in the proper stage of development. The age of the heads sampled was of primary importance in obtaining usable material. Approximately three days before emergence gave the most satisfactory material. This is best judged by pinching the boot to determine the relative stage of development of the head. However, some experience was required before an educated touch was developed. It was observed that the tendency was to sample too late rather than too early. This was indicated by the fact that a majority of the usable anthers were obtained from the upper and lower florets which are the later developing parts of the head, rather than from the earlier maturing middle portion of the head.

When the parent plants and crosses reached maturity they were harvested and immediately replanted, again utilizing greenhouse facilities as previously outlined. This material was seeded on May 12, 1961. Cytological investigation had been completed at this time on the monosomic parent material, but it was not possible to determine the chromosome number of all the parents sampled because of unsatisfactory PMC material in some cases. All of the crossed seed set on monosomic parents and also crossed seed from plants of unknown chromosome numbers were planted. This involved 183 F_1 seeds from a total of 55 crosses involving 20 of the 21 monosomic lines. Monosomic 7D had been lost because of mismatch of parents at heading

time. Monosomic 6A was subsequently lost in the F_1 generation because of failure to set seed. Also planted at this time were the seeds of the three normal KMC22 X Itana crosses, six seeds each of the monosomic and unknown KMC22 parents, each Itana plant used for crossing, and the three normal KMC22 plants. All F_1 plants from monosomic parentage were sampled for cytological investigation. Chromosomes 3B, 6B, and 3D could not be studied because of the failure to obtain appropriate monosomic F_1 plants. Plants from the monosomic and unknown KMC22 parents were sampled to insure an ample supply of monosomic parent seed of each chromosome for the final generation planting. Again, in some cases, a problem of not being able to make positive cytological identification was encountered because of unsatisfactory samples. All of the plants in this generation were allowed to self pollinate. The parent and F_2 seed from this greenhouse generation was planted in the field plot in the fall. However, because the May 15 planting date resulted in a late maturing greenhouse crop, the rather undesirably late dates of October 12-17 were obtained for the field planting. In an effort to realize as satisfactory a field planting date as possible, some of the greenhouse plants were harvested in a partially immature stage. This, combined with the late field planting, resulted in a considerable stand reduction in the field.

The field plot was located at the Montana State Experiment Station Field Research Laboratory west of Bozeman. The plot had been in alfalfa the previous summer, and in summerfallow the summer prior to planting. A sprinkler irrigation application of approximately two inches of water was

applied prior to seeding to insure adequate fall soil moisture. The rows were 18 feet long, spaced one foot apart, and each seed was planted six inches apart in the row. All seed was planted by hand with a hand corn planter.

The field plot design for each monosomic line included one row each of the Itana parent or parents utilized in crosses with the monosomic parents, the normal KMC22 and the normal Itana X KMC22 cross, and as many rows as seed was available for the monosomic and unknown F_2 populations and KMC22 parents. In some cases where adequate seed was available the material was replicated twice. In instances where there was not sufficient seed to complete the row it was finished by using an easily identifiable dwarf winter wheat as filler. The entire field experiment involved 784 rows. Following seeding, the plot was fertilized with the equivalent of 40 pounds of actual nitrogen and 20 pounds of phosphate per acre applied in granular form by spreader.

The following spring the plot was sprayed for broad leaf weeds and volunteer alfalfa with a knapsack sprayer application of 2,4D. Following this, the plot was kept as weed free as possible by hand hoeing. Because of adequate summer moisture, no irrigation water was applied. The entire plot was severely infested with stripe rust, Puccinia striiformis West., to which both parents were susceptible.

At the appropriate time morphological data were collected on the experiment. Heading date was determined as the date on which the first head of the plant emerged from the boot. Auricle color was classified

either as purple or colorless. Glume color was noted either as red or white.

At harvesting each plant was pulled and then threshed individually in the field with a Vogel head thresher. Power for the threshers was supplied by a portable Kohler power plant. Following threshing, the samples were cleaned of all chaff and other foreign material and stored in large coin envelopes.

The modified wheat meal fermentation time test as described by Laubach and Hehn5/ was utilized in the study of gluten quality. The samples were allowed to dry in storage for a minimum of 120 days after harvest to insure a relatively uniform moisture content of about 9 percent. Approximately 7.5 g. of whole wheat were weighed from each sample and ground in a Wiley mill equipped with a 1 mm. mesh sieve. The meal that passed through the sieve was collected and stored in small glass bottles with screw caps. Doughballs were made from this material approximately five to seven days following grinding. Preliminary investigations showed that 250 mm. low form beakers containing 100 mm. water would serve adequately for doughballs containing 5 g. of meal, without allowing any support to the doughballs by the sides of the beaker. The doughballs were made by using 5 g. of the wheat meal, 2.5 ml. of a solution of 60 g. Fleishmans yeast dissolved in 100 ml. water, 0.5 ml. of a solution of 5 g. glucose dissolved in 100 ml. of water, and 0.5 ml. of a solution of 1 g. potassium bromate dissolved in 500 ml. of water. Distilled water was

5/ Laubach and Hehn, op. cit.

used in all phases of this experiment. The meal and solutions were mixed together using a spatula and crucible. When the material formed a sticky mass it was transferred to the palm of the left hand and kneaded with the thumb of the right hand until a ball of medium consistency was formed. This was rolled between the palms until a smooth ball without cracks and crevices was formed. The finished product was placed in the 250 ml. low form beaker containing 100 ml. of distilled water. The water had previously been brought to a uniform temperature of 86°F by placing the beaker and water in a fermentation cabinet for about 90 minutes prior to the immersion of the doughball. It was possible, by using this fermentation cabinet, to maintain the desired temperature as a constant throughout the experiment. The cabinet also provided facilities to allow the fermentation of about 30 doughballs at the same time. By starting them on a rotational basis it was possible to utilize the entire cabinet until a particular run had been completed. Approximately 150 doughball tests could be completed in a 12 hour operation. The only humidity that was supplied was furnished by the water in the beakers. Time was measured in minutes from the immersion of the doughball to the point when the first piece of dough touched the bottom of the beaker. An attempt was made to reduce the error in the doughball testing by keeping all factors as constant as possible. These factors included the length of time from grinding to running, the temperature of the cabinet and water, and a minimum amount of disturbance to the doughball-water-beaker system.