Variation of seed storage protein profiles within spring wheat varieties
by Mark Edward McLendon

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University
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Abstract:
Bread-making capability of wheat (Triticum aestivum L.) is dependent on the seed storage proteins which consist of gliadins and glutenins. We have analyzed 25 widely grown or historically significant hexaploid spring wheat cultivars for intravarietal differences of gliadin and glutenin proteins. The objective of this study was to improve the quality of existing spring wheat cultivars by selecting for seed storage proteins and to test the effect of particular proteins on bread-making capability of spring wheat.

Twelve of the cultivars showed plant-to-plant variation in either their gliadin or glutenin makeup as revealed by SDS-PAGE. In 1990, a randomized split plot experiment with three replications was planted to determine if the protein variants were due to contamination or a biotype that naturally occurs within a cultivar, and to obtain enough seed for quality testing. Based on agronomic data, visual comparisons and gel profile ratios, we determined that 5 cultivars out of the original 25 contained biotypes.

A quality analysis was then conducted to compare bread-making capability of the biotypes with the original cultivar they were selected from. Very few significant differences were noted for quality between the biotypes and the control cultivar except in Wampum, which differed in HMW glutenin proteins. One of the biotypes, which contained a HMW glutenin subunit known to confer good quality, showed increased mixing and peak time, both indicators of dough strength.

A possible explanation for the lack of quality differences among biotypes is that our experiment was too small to detect any. We did not have enough seed to do the standard baking tests, which can cause problems with proper mixing of dough. A larger field experiment has been set up for 1991 which will give us a better indication of quality for the biotypes.

VARIATION OF SEED STORAGE PROTEIN PROFILES

WITHIN SPRING WHEAT VARIETIES

by

Mark Edward McLendon

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

viii

ABSTRACT

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is dependent on the seed storage proteins which consist of
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INTRODUCTION

Wheat provides man more protein than any other source. A kernel of wheat contains 9% to 16% protein, most of which is found in the endosperm in the form of storage proteins classified as prolamines. Prolamines play a major role in the type of end-use product produced from a particular cultivar of wheat. The prolamines consist of two types, gliadins and glutenins, that to a large extent determine the quality of wheat. They give bread dough made from flour the characteristics of extensibility and elasticity. The balance between these two properties is genetically determined and can vary greatly between cultivars. Cultivars that produce a strongly elastic dough with some extensibility are used for bread, while varieties producing dough with high extensibility are used for cookies.

The gliadins and glutenins have been studied extensively with the goal of manipulating the quality of wheat. A better understanding of how these proteins perform in food products could provide plant breeders with a useful tool to implement into a program. Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) has been the most widely used method to study these proteins. This technique separates proteins on the basis of molecular weight into a series of subunits that can be visualized on a gel. Some evidence suggests that these subunits play a key

role in the functional properties of the protein. Thus, a question of interest regards whether quality can be improved by selecting for specific subunits.

Spring wheat is an important economic crop in Montana, grown on 1,134,000 ha and producing 3,786,156,500 kg in 1990, mainly in the northeastern and northcentral parts of the state. Superior end-use properties are an important factor in the marketability of the crop. Montana has a dry, semi-arid climate that is conducive to producing a high quality product. In 1989, 90% of the spring wheat produced in the state was shipped to the Pacific NorthWest and then exported to Pacific Rim countries. Montana grown wheat is preferred because of its high quality.

We have found, using SDS-PAGE, that several spring wheat varieties are composed of 2-3 apparent biotypes based on seed storage proteins. These protein variants could have two explanations, either an actual biotype inherent within the variety, or a contamination from some unknown source. This provided an opportunity to test the variants, determine whether they were contaminant or biotypes, and then to establish pure lines for any actual biotypes. These biotypes should be genetically similar for all traits except seed storage proteins. The purpose of this research was to determine if the biotypes had different end-use properties compared to that of the original cultivar. This allowed us to test the value of specific subunits, and may also allow

derivation of superior wheat cultivar by establishing pure-lines of the storage protein biotypes.

REVIEW OF LITERATURE

Protein Composition

Wheat proteins are divided into four classes: albumins, globulins, gliadins, and glutenins (Osborne 1907). The albumins and globulins are mainly enzymes, rich in essential amino acids, but do not affect bread-baking properties. Hoseney et al. (1969) found that addition of these proteins to wheat flours showed no affect on the baking characteristics of the flours.

Two types of storage proteins are normally recognized in the endosperm, gliadin and glutenin, which together make up gluten. Gluten gives wheat dough its unique cohesive and elastic properties which allow it to be used for baking bread (Wall 1979). These proteins are synthesized on the endoplasmic reticulum and then deposited in protein bodies and provide a source of nitrogen for germinating seeds (Shewry and Milfin 1985). Originally, they were defined on the basis of solubility in alcohol, but now, are normally classified by their state of aggregation in dissociating media. Glutenins are considered to be aggregated storage proteins while gliadins are non-aggregated proteins occurring in free polypeptide chains (Jackson et al., 1983). Protein quality differences between cultivars is largely a function of different combinations of seed storage proteins (Payne et al., 1984).

Gliadin proteins are small molecules, approximately 35,000 molecular weight, and consist of a complex mixture of polypeptides. Fractionations of these polypeptides according to charge by SDS-PAGE causes separation into four groups: α, β, γ, ω (Wall 1979). A hexaploid wheat genotype will consist of about 25 major gliadin polypeptides and as many minor components (Wrigley and Shepherd 1973).

Glutenins are large molecules made from different subunits that are linked by disulfide bonds (Wall 1979). When exposed to a reducing agent, such as 2-mercaptoethanol, the disulfide bonds break and glutenin molecules dissociate into several subunits that are classified into two groups, high molecular weight (HMW) and low molecular weight (LMW) subunits (Payne and Corfield 1979). Storage proteins of wheat flour typically consist of 50% gliadin, 10% HMW glutenin subunits, and 40% LMW glutenin subunits.

Endosperm proteins are also useful for varietal identification. Jones et al. (1982) studied gliadin profiles obtained by SDS-PAGE of 88 wheat cultivars grown in the U.S. and found that 76 out of 88 showed unique profiles. Cultivars that had identical profiles were found to have very similar genetic backgrounds.

Genetics of the Endosperm Proteins

Wheat (Triticum aestivum L.) is a hexaploid species that contains three genomes of seven pairs of chromosomes

each. The genomes are designated A, B, and D. Each one of the 21 chromosome pairs is given a number, 1 to 7, followed by the appropriate genome designation (Payne 1987). Use of chromosome mutants has been exploited by cytogeneticists in genetic studies of wheat. Nullisomic and tetrasomic lines of Chinese Spring have been particularly helpful in studying storage proteins (Sears 1966). Genes that control high molecular weight (HMW) glutenin subunits are located on the long arm of chromosomes 1A, 1B, and 1D (Payne 1987). Loci that contain these glutenin genes are together called Glu-1, while individually labelled as Glu-A1, Glu-B1, and Glu-D1. Each genome in hexaploid wheat contains two HMW subunits, broken into x and y types based on higher or lower molecular weight, respectively. A cultivar will have six glutenin genes but will only synthesize three to five subunits, presumably because of gene inactivations. Gene locus Glu-A1 contributes one subunit whereas the other two loci normally, but not always, contribute a pair of subunits (Payne et al., 1981a; Payne et al., 1982; Anderson and Greene 1989).

Payne and Lawrence (1983) devised a standard labelling scheme for the HMW glutenin subunits using Chinese Spring and 15 other cultivars as standards (Fig. 1, Table 1). These standards consist of all subunits known to exist, except for several new ones found in landraces. Subunits from other cultivars can be compared to these standards and labelled with the appropriate locus followed by a small

letter. For example, cultivars that contain the 2 + 12 subunits from the D locus are defined as being <u>Glu-D1a</u>, while cultivars containing the 5 + 10 subunits are labelled as containing <u>Glu-D1d</u> (Table 1).

Gliadin proteins are synthesized by genes located on chromosomes of homological groups one and six. Genes that control ω gliadins, and γ gliadins occur on the short arms of chromosome 1A, 1B, 1D, and are collectively known as <u>Gli-1</u>. Genes for α and β gliadins are on the short arm of chromosomes 6A, 6B, 6D, and are collectively known as <u>Gli-2</u> (Payne et al., 1982; Payne 1987). Evidence suggests that gliadins are inherited in groups or specific blocks of components. Little recombination has been observed between the allelic variants of the blocks, suggesting the existence of clusters that are closely linked (Baker and Bushuk 1978; Sozinov and Poperelya 1980; Metakovsky et al., 1985, 1986; Payne et al., 1984). A few gliadin coding genes exist that are removed from the main cluster. These genes have been identified in a number of cultivars, including Chinese Spring (Branlard 1983; Galili and Feldman 1984; Metakovsky et al.,1986).

Low molecular weight (LMW) subunits of glutenin are encoded by genes on chromosomes 1A, 1B, and 1D (Payne 1987). Genes for LMW subunits are designated <u>Glu-3</u>, and have been shown to be very closely linked with the <u>Gli-1</u> locus (Singh and Shepherd 1988). Strong associations between the two

	--1
1D --2	--2*
1B --7	
1B --8	Gene Locus <u>Glu-A1</u>
1D --12	
	<u>a</u> <u>b</u> <u>c</u>
1D --2	--6
1B --7	--7 --7 --7 Gene Locus <u>Glu-B1</u>
1B --8	--8 --8
1D --12	--9
	<u>a</u> <u>b</u> <u>c</u> <u>d</u>
1D --2	--2 --3 --4 --5
1B --7	Gene Locus <u>Glu-D1</u>
1B --8	
1D --12	--12 --12 --12 --10
	<u>a</u> <u>b</u> <u>c</u> <u>d</u>
⇑	⇑
Chinese Spring	HMW glutenin standard subunits based
HMW subunits	on Payne (1983).
(Standard)	

Figure 1. - HMW glutenin variant subunits. This is a
partial list of subunits from Payne and Lawrence (1983)
showing only standard cultivars (Table 1) that we used to
mark our variant subunits. To number these subunits, they
were electrophoresed beside the standard cultivars and lined
up with the corresponding subunit.

Table 1. Partial listing of allele designations used in our
          study for the <u>Glu-A1</u>, <u>Glu-B1</u>, and <u>Glu-D1</u> loci as
          recommended by Payne and Lawrence (1983).

Locus	Allele Designation	Number Designation	Variety Standard
<u>Glu-A1</u>	a	1	Hope
	b	2*	Bezostaya-1
	c	-	Chinese Spring
<u>Glu-B1</u>	a	7	Flinor
	b	7 + 8	Chinese Spring
	c	7 + 9	Bezostaya-1
	d	6 + 8	Hope
<u>Glu-D1</u>	a	2 + 12	Chinese Spring
	b	3 + 12	Hobbit
	c	4 + 12	Champlein
	d	5 + 10	Hope

HMW glutenin subunits are designated first by the locus
which they appear in, followed by the allele designation.
A cultivar will contain one variant type from each of the
loci. For example, Hope contains the following subunits:
1 = <u>Glu-A1a</u>, 6 + 8 = <u>Glu-B1d</u>, 5 + 10 = <u>Glu-D1d</u>. Allele
<u>Glu-A1c</u> produces a non-detectable subunit and is considered
to be null.

have been noted (Payne et al., 1984; Autran and Galterio 1989). LMW glutenins have been difficult to study because, upon extraction in an SDS buffer and subsequent reduction, they have a similar mobility to gliadins using SDS-PAGE (Payne et al., 1984). This has been overcome by a two-step, one dimensional method that allows LMW subunits to be visualized free from other proteins (Singh and Shepherd 1985, 1988).

Allelic Variation

Although only nine major gene loci code for seed storage proteins, considerable allelic variation still exists between these proteins (Payne 1987). When 300 wheat cultivars were analyzed for HMW glutenin variation, 3 alleles for Glu-A1 were identified, 11 alleles for Glu-B1, and 5 alleles for Glu-D1 (Payne and Lawrence 1983). Abundant allelic variation also exists for gliadins (Metakovsky et al., 1984). Thus, the total number of possible combinations is large, approaching 500 million. This allelic variation is the basis for studying quality differences between wheat cultivars.

HMW Glutenin and Quality

Payne et al. (1979, 1981a) were the first to report an association between HMW subunits and flour quality, particularly glutenin subunits 5 + 10 and 2 + 12, which are

associated with resistance to dough extension and dough
weakness, respectively. These results have been
substantiated by several other studies using different
methods and genetic materials (Payne et al., 1984, 1987b;
Branlard and Dardevet 1985; Lawrence et al., 1987, 1988;
Mansur et al., 1990).

Branlard and Dardevet (1985) found that HMW subunits
2*, 5 + 10, and 7 + 9 were positively correlated with gluten
strength and tenacity, while subunits 1, 13 + 16, 17 + 18
were positively correlated with dough extensibility.
Lawrence et al. (1987) examined biotypes derived from within
a variety and found the greatest contrasts were found
between pairs of biotypes differing at the Glu-D1 locus for
5 + 10 and 2 + 12. Lawrence et al. (1988) also studied
lines that were deficient in glutenin subunits expressed by
the three loci and found that the loss of 5 + 10 from Glu-D1
and 17 + 18 from Glu-B1 had greater effects than the loss of
subunit 1 at Glu-A1 locus.

Overall, most studies support the idea that the 2*
subunit at locus Glu-A1 is superior to subunit 1, and at
locus Glu-D1, 5 + 10 subunits are superior to 2 + 12 for
wheat flour quality. However, the Glu-B1 locus has shown no
clear correlations, with some work showing positive results
and others showing none (Anderson and Greene 1989). Payne
et al. (1984) concluded bands 7 + 8, 13 + 16, and 17 + 18
were equivalent in quality contributions. Branlard and

Dardevet (1985) found positive correlations for dough quality in alleles 7 + 9, 13 + 16, and 17 + 18. Grama et al. (1987) rated the 7 + 8 allele as having the highest quality and 7 + 9 with the lowest. Dougherty et al. (1990) used two dimensional electrophoresis to study baking quality factors. They found that two protein spots, considered to be HMW glutenins coded by chromosome 1B, had negative correlations for mixograph mixing time. Protein spots thought to be associated with chromosome 1D showed no significant relationships with loaf volume or mixing time.

Flavell et al. (1989) studied the amino acid sequence of subunits 10 and 12 and found relatively few differences between them. The nonrepetitive regions at the N and C termini were identical, suggesting that differences in dough property between the two are from an array of 6 to 9 residue repeats found in the central segments of the proteins. A possibility they suggested was that subunit 10 had more of the amino acid repeat units, leading to longer regions of predicted β turns which could give better elastic dough properties.

Cysteine residues, which form the intermolecular disulfide linkages, are thought to be important in dough structure (Flavell et al., 1989). Anderson et al. (1989) found that subunit 5 had an additional cysteine residue when compared to subunit 2. Subunit 2*, which is associated with good quality, also lacked the cysteine residue. Another

difference was that a <u>Glu-A1</u> subunit had an increased charge density while one <u>Glu-B1</u> subunit had a different repeat motif. No conclusive evidence has been found to show that these factors directly affect quality, but they may be areas worthy of further study.

Gliadin and Quality

The presence of certain γ-gliadins have been shown to be correlated to flour quality (Damidaux et al., 1978; Kosmolak et al., 1980; du Cros et al., 1983). Durum wheat, which lacks the D chromosome and contains no <u>Glu-D1</u> alleles, has been studied extensively. Most of the allelic variation of subunits is determined by the <u>Gli-B1</u> locus which codes for the γ-gliadin (Autran and Galterio 1989). Gliadin subunit γ-45 (nomenclature of Bushuk and Zillman, 1978) is associated with increased elasticity and dough strength, while gliadin subunit γ-42 is associated with poor viscoelastic properties and dough weakness (Kosmolak et al., 1980; du Cross et al., 1982).

Branlard and Dardevet (1985) tested seventy wheat cultivars with different genetic backgrounds and quality characteristics using several quality tests. They found eighteen bands correlated with at least one aspect of quality. Ten correlations were positive and 11 correlations were negative.

Campbell et al. (1987) noted that gliadin bands 58, 59, 60, and 67, presumably coded by the group six chromosomes (Gli-2), were strongly associated with dough resistance. These bands have received little attention, probably due to poor resolution by most electrophoretic procedures.

LMW Glutenin and Quality

With the advent of a two-step, 1-dimensional SDS-PAGE procedure (Singh and Shepherd 1985, 1988), it was revealed that LMW glutenin had extensive variation in both bread wheats and durum wheats (Gupta and Shepherd 1987, 1988). In the first step of the technique proteins are extracted in hot ethanol and then electorphoresed at a high voltage without exposing the proteins to a reducing agent. This allows gliadins to migrate down the gel and leave HMW and LMW glutenins at the top. The top first cm, containing two types of glutenins is cut off, reduced, and applied on top of a second polyacrylamide gel and electrophoresed. This allows visualization of LMW glutenins free from the presence of the gliadin proteins.

Since the evidence suggests that LMW glutenin are closely linked to the Gli-1 locus (Singh and Shepherd 1988), which encodes gliadin bands 45 and 42 in durum wheat, these glutenins could be the cause of quality differences noted (Autran et al., 1987; Gupta et al., 1989; Pogna et al., 1988). The first evidence of this was given by Moonen et

al., (1982), who used the SDS-sedimentation test to screen wheat varieties and found the Gli-1 locus positively correlated with bread-baking. Sedimentation tests measure quality as a function of glutenin not gliadin, thus suggesting LMW subunits were involved. More recent studies have shown that allelic variation of LMW subunits are significantly correlated to flour quality in both hexaploid and durum wheats (Payne et al., 1984; Autran et al 1987; Gupta et al., 1989). Pogna et al. (1988) studied a durum wheat variety that contained only gliadin band 42 and LMW subunits and found that this line had quality equal to high quality varieties containing gliadin band 45 and LMW subunits. This showed that the LMW subunits were likely responsible for the increased quality in the durum variety instead of gliadin band 45. Gupta et al. (1989) showed that a specific Glu-3 band (Glu-A3m) was positively correlated with dough resistance and extensibility at two different protein levels (7% and 14%) and had a larger affect on quality than the Glu-A1b HMW allele. Payne et al. (1987a) found that a different LMW glutenin subunit, Glu-A3a had positive effects on bread quality as measured by the sedimentation test and extensometer. Thus, evidence suggests that LMW glutenins are more responsible for quality differences than gliadins and need to be studied more extensively.

Evidence of Biotypes

A biotype is defined as a naturally occurring variation, like storage proteins, found within a cultivar that is genetically similar for all other traits. Lawrence et al. (1987) found a number of Australian wheat cultivars that contained two or three storage protein biotypes. This gave them the opportunity to establish pure lines within cultivars that were likely to be genetically similar, except for seed storage protein composition, and to compare their qualities. Of ten cultivars found to contain biotypes, all showed a difference in HMW glutenin subunits but, except in one case, were identical for gliadin makeup. Their findings showed that the main difference in quality occurred at the Glu-D1 locus with the 5 + 10 subunit conferring greater dough resistance than the 2 + 12 subunit. Only small differences were noted between biotypes at the Glu-A1 and Glu-B1 loci.

Gupta and Shepherd (1990) used the two step-one dimensional SDS-PAGE technique to analyze LMW glutenin subunit composition of 222 hexaploid wheats. They found that thirteen out of the 222 cultivars studied consisted of two biotypes with regards to LMW subunits. Five of the biotypes were grown to maturity and were found to have similar or identical plant morphological characteristics and thus were determined to be actual biotypes and not contamination due to seed mixture or outcrossing.

In conjunction with our spring wheat work, we examined eighteen common North American winter wheat varieties using single kernel analysis with SDS-PAGE for within cultivar variation of seed storage proteins. It was determined that six cultivars consisted of two apparent biotypes each. Data is from gel profiles only, agronomic and visual inspections have not been done. Four cultivars differed in their HMW glutenin subunits while two showed a difference in the gliadin or LMW glutenin profile (McLendon and Kisha, unpublished).

MATERIALS AND METHODS

Cultivar Selection

Twenty-five spring wheat cultivars were chosen on the basis of being commonly grown or having historical significance to Montana. The cultivars used in the study were Alex, Amidon, Borah, Butte, Coteau, Era, Fortuna, Glenman, Lew, Len, Marquis, MT 8402 (Hi-Line), MT 8561, Pondera, Newana, Norana, Olaf, Oslo, Protor, Rambo, Sheridan, Thatcher, Tioga, Waldron, and Wampum. Seed was obtained from stocks maintained by the spring wheat breeding program at Montana State University.

Protein Extraction

Twenty seeds were selected at random from each cultivar, surface sterilized, cut in half, and the embryo portion stored at 4 o C. Seed storage proteins were extracted from the brush end of the seed based on the method of Laemmli (1970). Individual endosperm portions were crushed with a pestle, placed in an eppendorf tube, and suspended in 500 ul of 70% ethanol. Samples were vortexed, incubated at 55 o C for one h, spun for two min at 10K on a eppendorf micro-centrifuge, and 250 ul of supernatent was removed. Supernatents were then placed at 4 o C for one h, dried in a centrifuge under vacuum, and reduced in cracking buffer.

Recipes for all reagents are in the Appendix (Table 9).
Samples were then placed at 4 o C until loading time.

Electrophoresis of Proteins

Protein samples were fractionated using single kernel
analysis by SDS-PAGE, based on the method of Laemmli (1970),
and improved by Payne et al. (1981a). Electrophoresis was
through acrylamide gels consisting of a 6% stacking gel and
a 12% resolving gel, each one centimeter thick. After
boiling, $20\mu\ell$ from each sample was loaded into individual
gel slots and then electrophoresed for 16 h at 80 v. Each
gel contained 20 individual seeds selected from a cultivar.
Gels were stained for two h in coomasie blue, destained
until protein bands were visible, and dried down on 3mm
Whatman paper under vacuum.

Selection of Biotypes

Gels were visually examined for variation in the
gliadin or glutenin protein subunits within a cultivar. If
variants were noted within a cultivar, then embryos were
matched to the correct gel profile. Embryos were placed in
an eppendorf tube holder containing a wet kimwipe and
allowed to germinate. Seedlings were transplanted
individually to pots in the greenhouse where two generations
were grown. Seeds from like profiles within a cultivar were

bulked from each variant, giving enough seed for a field
experiment.

Experimental Design

Variants were planted at Bozeman in the spring of 1990.
The design was a randomized split plot experiment with three
replicates, with cultivars as main plots and variants as
subplots. Subplots consisted of variants planted in plots
of one row, and a control consisting of the original
cultivar. Seed was planted in 3.04 m rows, 2.63 g/m and
later trimmed to 2.43 m at harvest. Data collected
throughout the growing season included heading date (days),
plant height (cm), stem solidness (5-25) with 5 being hollow
and 25 being solid, and yield (kg/ha). Biotypes were also
visually compared to the control for morphological
characteristics. Plots were harvested in August for baking
quality analysis.

Quality Analysis

Baking analysis was determined with the help of
personnel in the Cereal Quality Laboratory, Montana State
University, Bozeman, Montana. Grain samples were first
tempered overnight and then milled with a Buhler Laboratory
mill (American Association of Cereal Chemists (AACC), 1983
method 26-10). Grain protein was determined using Near
Infrared Reflectance (NIR) (Williams, 1979). Approved

methods of the AACC were used for the straight-dough method
of baking quality analysis, modified to a 25g sample (AACC,
1983 method 10-10b). The following data were recorded for
each flour sample.

- Grain protein (%)

- Water absorption (%) - the amount of water estimated
  from the mixograph curve.

- Peak Time (converted from cm - paper units) - the
  time from the first addition of water to the doughs
  maximum consistency.

- Peak height (converted from cm - paper units) -
  distance in mixograph units when curve reaches
  maximum height.

- Bake Absorption (ml) - The amount of water added to
  prepare flour for baking.

- Loaf volume - measured using the rape seed
  displacement

- Crumb score - determined on a scale from 1 to 10
  (1 = poor and 10 = excellent) by visual comparison
  with standard loaves (baked from commercial flour
  Mello Judith).

- Mixing time (minutes) - the time needed to bring the
  dough to optimum consistency.

22

Statistical Analysis

Data were subjected to analysis of variance for each measured variable. The main interest was to compare variants and control within each cultivar rather than comparing cultivar means. Variants and control within a cultivar were compared using the Least Significant Difference (LSD). The subplot error mean square and degrees of freedom was used in computing the LSD. Analysis of variance tables are listed in the appendix (Tables 10 and 11).

RESULTS AND DISCUSSION

Identification of Storage Protein Biotypes

Of the twenty-five cultivars that were analyzed with
SDS-PAGE, twelve showed within cultivar variation for
storage proteins. These twelve cultivars were analyzed to
determine if protein variants were actually biotypes or an
unknown source of contamination. A biotype is defined as a
naturally occurring variant within the cultivar for storage
proteins. However, the possibility existed that variants
were a result of contamination that occurred from
outcrossing or a different seed source. Each variant was
compared to the original cultivar for morphological
characters, both visually and agronomically, and protein
profiles from polyacrylamide gels were compared using SDS-
PAGE (Table 2). If a profile occurred only once in 20 seeds
examined from a cultivar during electrophoresis, it was
suspected to be a contamination. Profiles that occurred
multiple times were candidates to be actual biotypes. The
following description of the twelve cultivars refers to
Table 2.

The cultivar Borah was determined to consist of two
storage protein biotypes. Visual comparisons and agronomic
data measured in the field showed no differences between
biotypes and the control cultivar. Biotype profiles

Table 2. Agronomic comparisons between storage protein
variants and the original cultivar.

Biotypes		Heading Date days	Stem Solid 5-25	Yield kg/ha	1000 Kernel Weight g	# Seeds /Biotype
Borah	A	186.0 A	10.3 A	2324 A	30.0 A	18/30
	B	186.0 A	10.7 A	2291 A	30.7 A	12/30
	C	185.7 A	11.3 A	2143 A	29.8 A	
Butte	A	186.0 A	7.2 A	2438 A	31.3 A	19/20
	B	187.0 B	18.9 B	2002 A	31.4 A	1/20
	C	186.3 AB	7.8 A	2197 A	30.5 A	
Fortuna	A	188.0 A	20.4 BC	1404 A	41.0 C	19/35
	B	187.7 A	22.3 C	1552 A	38.4 B	15/35
	C	187.7 A	17.5 AB	1747 AB	39.7 BC	
	D	188.8 A	15.7 A	2264 B	30.8 A	1/35
Lew	A	189.0 A	9.8 A	3232 B	33.4 A	1/20
	B	189.3 A	15.4 B	2298 A	33.2 A	19/20
	C	185.7 A	15.1 B	2264 A	35.1 A	
MT8561	A	190.7 A	8.1 A	2345 A	28.6 A	1/20
	B	190.7 A	8.3 A	2345 A	27.8 A	19/20
	C	190.7 A	9.3 A	2177 A	28.6 A	-
Norana	A	189.0 A	9.5 A	2977 B	31.2 A	4/20
	B	188.0 A	9.8 A	2271 A	30.4 A	16/20
Olaf	A	188.0 A	9.1 A	2499 A	36.2 A	4/20
	B	188.3 A	9.3 A	2459 A	35.3 A	16/20
	C	187.7 A	8.7 A	2177 A	34.9 A	
Pondera	A	189.3 B	7.4 A	2600 A	30.5 B	2/20
	B	186.0 A	8.4 A	2177 A	27.9 A	17/20
	C	186.3 A	8.4 A	2278 A	29.3 AB	
	D	187.0 A	7.1 A	2163 A	30.7 B	1/20
Sheridan	A	189.7 A	8.4 A	3003 B	32.0 A	22/32
	B	189.7 A	7.5 A	2204 A	31.6 A	10/32
	C	189.0 A	7.9 A	1854 A	30.9 A	
Thatcher	A	188.0 A	8.8 A	2305 A	26.6 A	22/33
	B	188.0 A	6.4 A	2103 A	25.6 A	11/33
	C	187.0 A	6.7 A	1968 A	25.7 A	
Unknown	A	185.0 A	10.1 A	2506 A	31.4 A	8/20
	B	185.0 A	8.8 A	2277 A	31.6 A	12/20
	C	185.7 A	9.9 A	2378 A	32.2 A	
Wampum	A	189.7 A	7.4 A	2855 AB	33.5 A	8/34
	B	189.7 A	9.6 A	2660 AB	31.9 A	12/34
	C	189.7 A	7.0 A	3184 B	31.6 A	
	D	190.0 A	8.1 A	2586 A	32.7 A	8/34

Means are compared within individual cells. Means followed
by the same letter are not significantly different at the
0.05 level by LSD. C = control variety. Stem solidness -
5 = hollow and 25 = solid.

showed up on a polyacrylamide gel in a ratio of 12 to 8 for biotypes A and B, respectively.

The cultivar Butte contained no biotypes. We suspected that Butte contained a contaminant from the gel profile ratio of 19 to 1 for the two variants. Visual comparison and agronomic data confirmed this. The B variant was awnless compared to the normal Butte cultivar, which is awned. A large difference was noted for stem solidness (18.9 - B, 7.8 - Control). The Butte B variant was determined to be due to contamination and the other variant, Butte A, is the actual variety.

Fortuna had three variant profiles that showed up on a gel in a ratio of 11 A, 8 B, to 1 for D (suspected contaminant). Visual inspection in the field showed that A and B were morphologically similar to the control while D had a lighter head color. The agronomic data was harder to interpret. Variant B showed no significant differences for the control, but A differed for stem solidness and D differed for yield. A difference also occurred between the A and B variants for kernel weight. Variant D was determined to be a contamination based on the gel profile ratios, the lighter head color, and yield difference. To further test that A and B were true biotypes we obtained certified Fortuna seed from the Montana seed certification program. This seed showed the same two variants close to the same ratio observed previously. Based on this

information, Fortuna was determined to consist of two biotypes.

The cultivar Lew was determined to contain no biotypes. The ratio for the gel profile was 19 A to 1 for B. When grown in the field, variant B had a large club head compared to a slender, smaller head for the control. Large differences also occurred in stem solidness and yield. Variant B looks like a type of white wheat and is a contaminant in the cultivar Lew.

The results from MT8561 are difficult to interpret. The gel profiles showed a ratio of 1 A to 19 B, suggesting a contaminant, but visual inspection and agronomic data showed no differences when compared to the control. The original selections were made from a gel with poor resolution. A different gel, using seed from three generations later, showed that A, B and the control cultivar had identical profiles, suggesting the original selection was a mistake. Either MT8561 has two biotypes, one in a very low ratio, or it contains no biotypes. Data suggests the latter to be true.

The cultivar Norana contained no biotypes. Even though the original selections showed up in a ratio of 3 A to 17 B, a polyacrylamide gel using material three generations later failed to show variant B. Variant B also showed a large difference in yield compared to A.

The cultivar Olaf contains no biotypes or contaminants. No differences were noted with the visual inspection or agronomic data between the two selections. The original gel profiles showed a ratio of 3 A to 17 B, but the gel was poorly resolved. A gel done three generations later showed no variation between variant A, B, or the control. The selection which was made from a poorly resolved gel, and thought to be a variant, was actually the same as the other profiles.

Pondera contained no biotypes. The gel profile showed a ratio of 2 A, 17 B, and 1 D, suggesting two contaminants present at low ratios. Significant differences were also noted for A and D for several agronomic characteristics. A gel consisting of a different seed source, certified Pondera, showed only the B profile. Variants A and D were contaminants while B was the actual original variety.

Sheridan contained two biotypes. The gel profiles had a ratio of 13 for the A variant and 7 for B. No differences were observed either for visual inspection or agronomic data, except that variant A out-yielded the control by 1142 kg/ha. This could be due to a genetic factor that links yield to the protein variation. It will be interesting to see if this observation holds true next year with a larger field experiment.

The cultivar Wampum was determined to have three biotypes with a possible fourth. The gel ratios were, 5 for

the A variant, 8 for B, and 5 for the D variant. A fourth

variant, which was present twice in the original gel but was

missed, showed up in approximately 27% (10/40) of the

profiles in a gel of the control Wampum done three

generations later.

An unknown cultivar was determined to have two

biotypes. The cultivar was originally thought to be Len,

but when it was electrophoresed next to certified Len on a

gel, its profile was different. The cultivar was

mislabelled and its true identity is unknown. Gel profile

ratios were, 8 for the A variant and 12 for B. No

differences were noted for visual inspection or in agronomic

data.

In summary, 12 of the 25 cultivars we studied

contained variants. Of these 12 cultivars, 5 were

determined to consist of biotypes.

Quality Evaluation of Biotypes

The following observations are based on data from the

quality analysis done on biotypes (Table 3).

The cultivar Borah contains two biotypes (Figure 2),

which consist of the gluten subunits shown in Table 4.

Biotypes had the same HMW glutenin subunits but varied in

their gliadin or LMW glutenin makeup. Neither biotype

showed significant differences for any quality factors

measured.

Table 3.   Quality data for storage protein biotypes.

Biotype		Protein %	Water Absorption %	Peak Height cm	Peak Time cm	Bake Absorption ml	Mix Time min.	Loaf Volume cc	Crumb Score
Borah	A	14.0 A	70.2 A	7.2 A	3.2 A	73.5 A	5.0 A	260.0 A	4.3 A
	B	14.1 A	69.9 A	7.2 A	3.4 A	74.0 A	4.8 A	265.0 A	3.7 A
	C	14.4 A	69.4 A	7.0 A	3.3 A	73.3 A	4.9 A	253.0 A	3.7 A
Fortuna	A	17.1 C	75.4 A	8.9 A	2.9 A	78.3 A	5.1 A	270.0 A	3.7 A
	B	17.3 C	75.4 A	8.9 A	2.8 A	78.4 A	5.0 A	263.3 A	3.0 A
	C	16.2 B	73.6 A	8.3 A	3.1 A	78.1 A	4.3 A	261.7 A	3.3 A
	D	15.3 A	73.6 A	8.3 A	2.9 A	78.3 A	4.7 A	271.7 A	3.0 A
Sheridan	A	16.0 A	69.3 A	7.0 A	2.9 A	75.8 A	5.4 A	246.7 A	3.3 A
	B	15.9 A	70.3 A	7.3 A	2.9 A	76.3 A	5.2 A	263.3 A	3.7 A
	C	16.0 A	70.7 A	7.4 A	3.2 A	76.1 A	5.2 A	253.3 A	4.3 A
Thatcher	A	15.2 A	69.5 A	7.0 A	3.5 A	70.7 A	5.5 A	270.0 A	4.3 A
	B	15.1 A	69.3 A	6.9 A	3.2 A	70.9 A	5.0 A	261.7 A	4.3 A
	C	15.2 A	69.4 A	7.0 A	3.3 A	70.6 A	5.0 A	273.3 A	4.0 A
Wampum	A	13.2 AB	69.6 A	7.1 A	3.3 A	70.3 A	5.7 A	275.0 A	4.3 A
	B	13.0 A	69.5 A	7.0 A	3.4 A	69.2 A	5.6 A	271.7 A	4.7 A
	C	12.6 A	70.5 A	7.5 A	3.6 A	70.9 A	5.7 A	265.0 A	4.0 A
	D	13.8 B	69.2 A	6.9 A	4.5 B	69.8 A	6.7 B	255.0 A	4.0 A

Means are compared within individual cells only. Means followed by the same letter are not significantly different at the 0.05 level by LSD.

Table 4.   HMW subunits for Borah biotypes.

Variety	HMW Subunits 1/	Gliadin/LMW Variation
Borah A	Glu-A1b (2*) Glu-B1c (7 + 9) Glu-D1d (5 + 10)	Yes
Borah B	Same as above	Yes

1/   Based on method of Payne and Lawrence (1983).

Fortuna contains two biotypes (Figure 2), which contain glutenin subunits shown in Table 5. The biotypes had the same HMW glutenin subunit composition, but differed in either gliadin or LMW glutenin makeup.

Table 5.   HMW subunits for Fortuna biotypes

Variety	HMW Subunits 1/	Gliadin/LMW Variation
Fortuna A	Glu-A1b (2*) Glu-B1a (7 + 8) Glu-D1a (5 +10)	Yes
Fortuna B	Same as above	Yes

1/   Based on method of Payne and Lawrence (1983).

No differences were noted between the two biotypes for any quality factors except that both A and B showed a higher protein concentration than the control cultivar (Table 3). This difference corresponds with the yield data, where both types had a lower yield (201-268 kg/ha), though it was not significantly different from the control (P < 0.05).

, Sheridan contains two biotypes (Figure 3), which show variation in HMW glutenin profile, but not the gliadin/LMW glutenin profile (Table 6). No significant differences were noted for any quality factors measured (Table 3). Biotype A

also had the same level of protein as the control, though it had a much higher yield (1142 kg/ha).

Table 6. HMW subunits for Sheridan biotypes.

Variety	HMW Subunits 1/	Gliadin/LMW Variation
Sheridan A	Glu-B1d (6 + 8) Glu-D1c (5 + 10)	No
Sheridan B	Glu-B1b (7 + 8) Glu-D1c (5 + 10)	No

1/ Based on method of Payne and Lawrence (1983).

Thatcher contained two biotypes (Figure 3), which show no difference for the HMW glutenin profile but do vary in the gliadin/LMW glutenin profile (Table 7). No significant differences were noted for quality between the biotypes or the control for any factors measured (Table 3).

Table 7. HMW glutenin subunits for Thatcher.

Variety	HMW Subunits 1/	Gliadin/LMW Variation
Thatcher A	Glu-A1b (2*) Glu-B1c (7 + 9) Glu-D1d (5 + 10)	Yes
Thatcher B	Same as above	Yes

1/ Based on method of Payne and Lawrence (1983).

Wampum contained three biotypes with a possible fourth that was not selected for (Figure 4). Three biotypes differed in their HMW glutenin profiles but not for the gliadin/LMW glutenin profile (Table 8).

Table 8.   HMW glutenin subunits for Wampum biotypes.

Variety	HMW Subunits 1/	Gliadin/LMW Variation
Wampum A	Glu-B1c (7 + 9) Glu-D1a (2 + 12)	No
Wampum B	Glu-A1a (1) Glu-B1c (7 + 9) Glu-D1a (2 + 12)	No
Wampum D	Glu-A1a (1) Glu-B1c (7 + 9) Glu-D1a (5 + 10)	No

1/   Based on method of Payne and Lawrence (1983).

Wampum D was the only biotype to show differences for quality factors analyzed.   Biotype D had an increased peak time and mix time when compared to the control and other two biotypes.   This indicates that D has superior dough strength.   The D biotype contains high quality 5 + 10 subunits while the other two biotypes have poorer quality 2 + 12 subunits (Payne et al., 1979, 1981a, 1987b; Branlard and Dardevet 1985; Lawrence et al., 1987, 1988; Mansur et al., 1990).   This finding is consistent with previous studies.   Biotype D also had a significantly higher protein level than the control.   This has not been reported before in other studies involving biotypes.

Of the three cultivars (Borah, Fortuna, and Thatcher) which showed variation in either their gliadin or LMW glutenin proteins but not in HMW glutenins, none showed significant differences for quality factors examined.   The two step-one dimensional electrophoresis has not been done yet to determine whether these three biotypes differ in

their gliadin or LMW glutenin makeup. Several possible explanations for this result are, first, the variation in profiles for these biotypes is due to gliadins, which may have no effect on quality. This has been suggested in several other studies in which data have shown that LMW glutenins affected quality, though gliadins did not (Moonen et al., 1982; Pogna et al., 1987). A strong possibility however exists that at least one of the biotypes differs in its LMW glutenin makeup and that this difference had no effect on quality. Second, this experiment may have lacked precision to detect differences among biotypes. The equipment in the cereal quality lab is set up to do 100 gram samples but we had only 25 grams of flour. Steps were taken to minimize problems that might arise due to this discrepancy, but the risk is higher of getting poor mixing, resulting in dough that is not developed properly, which can affect measurement of quality factors.

Of the two cultivars which varied for HMW glutenin subunits but not for gliadins, one showed quality differences and the other did not. Wampum was discussed earlier and did show a quality difference between biotypes. The Sheridan biotypes varied at the Glu-B1 locus but showed no differences. Again, there are several possible explanations. First, the difference between the two biotypes, 6 + 8 compared to 7 + 8 subunits, has no affect on bread baking quality. Other studies have found conflicting

results concerning the <u>Glu-B1</u> locus. Payne et al. (1984) rated subunits 7 + 8, 13 + 16, and 17 + 18 equivalent and Branlard and Dardevet (1985) found positive correlations for 7 + 9, 13 + 16, and 17 + 18. However, Gramma et al. (1987) rated the 7 + 8 allele highest for quality and 7 + 9 the lowest while Dougherty (1990), using two dimensional electrophoresis, found protein spots thought to be from the 1B locus that had negative effects. A second possibility for the lack of observed quality differences is that our experiment may not have the precision necessary to detect any.

One rather surprising finding from this experiment is the lack of purity we found in the seed stocks maintained by our spring wheat breeding program. This shows how useful SDS-PAGE can be for identifying sources of contamination in a breeding program. The Lew A variant, determined to be a contaminant in this experiment, has become a problem in several certified spring wheat fields in the northeastern part of the state. By being able to identify and then visualize this contaminant in the field, the seed certification program can now determine its source and take steps to purify Lew.

Another example is Len, which was originally thought to contain two biotypes. However, when electrophoresis was performed with our seed source evaluated next to certified Len, the profiles did not match. Our seed source, thought

to be Len for at least the last five years, is actually something else. We have been unable to identify the seed source, but it is definitely not Len. This suggests that it would be worth while for plant breeders to check their seed stocks for proper labelling. By checking only 25 varieties maintained by our breeding program with SDS-PAGE, two serious mistakes were identified that have been now corrected.