Improving quality of hard white wheat (HW) cultivars in the Pacific Northwest is important to expand exports to Asia. Asian food processors prefer HW grain that can satisfy an array of baking needs, as well as produce superior quality noodle products. Three experimental populations were developed from single cross and backcross combinations of four parents with contrasting protein composition, bread-making attributes, and noodle quality. Three hundred-sixty $F_3$-derived $F_5$ progeny grown in Corvallis, Oregon in 2004 were evaluated for protein content, single kernel characteristics, milling yield, dough mixing properties, SDS sedimentation volume, and polyphenol oxidase activity. Variation in flour protein composition was determined by SE-HPLC and SDS-PAGE. The populations and progeny showed wide variations in quality. Ranges in protein composition and mixing properties exceeded those of the parents. Backcrossing had a significant influence on quality characteristics of progeny. Progeny with the $Glu-D1$ HMW glutenin subunit (GS) pair 5+10 had increased SDS sedimentation volume, mixing time, and tolerance as compared to those with HMW-GS 2+12. At the $Glu-B1$ locus, HMW-GS 7+9 was
associated with improved protein quality and mixing properties as compared with either 7+null or 6+8. The Glu-$A_1$ HMW-GS 1 was associated with increased mixing time and tolerance as compared with 2*; both of which had improved mixing properties as compared with progeny carrying the null allele at this locus. Changes in relative proportions of polymeric and monomeric proteins were associated with variation in HMW subunit composition. Five LMW-GS were found to be polymorphic in parents and progeny. The LMW-GS designated band-$a$, which had relatively higher molecular weight, was associated with increased protein quality and stronger dough handling properties. LMW-GS bands $b$ and $e$ were associated with reduced mixing time and tolerance and lower levels of monomeric proteins. Interactions of HMW and LMW subunits were generally associated with changes in magnitude of effects rather than change in rank. Influences of LMW-GS were more pronounced in progeny which carry HMW-GS associated with reduced dough strength, such as 2+12, 7+null, or 2*. Manipulation of protein composition can be an effective method to improve end-use quality of HW cultivars in the Pacific Northwest.
The Relationship of Protein Composition to End-Product Functionality of Hard White Wheat

by
Sarah B. Gehlhar

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degree of
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Sarah B. Gehihar, Author
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DEDICATION

To

my parents Mark and Joan

and

in memory of Mark G.
Introduction

The development of hard white wheat (HW) cultivars suitable for production in the Pacific Northwest (PNW) would be beneficial in expanding exports to Asia. Wheat imports in Asian countries have grown an average of 1.1%, annually, as the production potential is limited in these countries and can not meet growing population demands (FAPRI, 2006). Ease of access to the Asian market and high demand for HW in the Asian marketplace are major incentives to develop PNW adapted HW cultivars with superior end-use quality.

Staple foods in Asia, such as noodles and steambreads, are primarily made from HW flour (Lin and Vocke, 2004). Discerning Asian wheat buyers prefer HW for manufacturing of noodles because of brighter flour and product color (Hatcher and Kruger, 1993). Up to 45% of wheat flour in Asia is consumed in the form of noodles (Miskelly, 1996). Asian standards for food quality are very high; special attention is given to product color and texture, particularly in noodles and bread products (Ross, 2003). The key to being a successful supplier to the Asian wheat markets is developing cultivars that impart light dough color, color stability, and appropriate textural characteristics (Lin and Vocke, 2004).

Currently, Asia imports most of its HW from Australia, where HW production is well established, more abundant, and the cultivars have been tailored to the Asian markets (Lin and Vocke, 2004). Ultimately, Asian food producers would like to import a “dual-purpose” wheat that can be milled primarily for noodle flour and use secondary flour streams for bread flour. The Australian HW is not
considered dual-purpose. The same wheat cannot be efficiently used to produce both noodles and the sponge and dough breads produced in north and southeast Asian countries (Lever et al., 2005), except as a component in blends. Inadequate protein strength has been the primary disadvantage of the Australian HW cultivars. While US hard red spring (HRS) and hard red winter (HRW) wheat are generally recognized for their superior protein quality for bread products, their weakness is poor color integrity exhibited when used in Asian noodle products (Park et al., 1997).

A successful U.S. HW export market will depend on the ability to produce and market grain with optimized protein content and composition, end-product functionality, and color that meets multiple product uses. Protein functionality (quality) is based on the protein composition as determined by the presence or absence of specific high molecular-weight glutenin subunits (HMW-GS) and the relative proportions of HMW-GS, low molecular-weight glutenin subunits (LMW-GS), gliadins, and small molecular weight albumin and globulin components per unit of protein (Wrigley and Bietz, 1988). The relationship between protein composition and bread quality is well defined (Færgestad, et al., 2000; Baenziger et al., 2001; Peña et al., 2005). Yet, the optimal combinations of HMW and LMW-GS or ratios of major protein components that are needed to provide superior functionality for both noodle and bread products remains relatively unexplored.

Oregon HW cultivar development has been hindered by a genetic background which imparts weaker than desired dough characteristics. This in turn leads to poor loaf volume (breadmaking) potential. Improving protein composition of HW
cultivars for the PNW is crucial, as the environment makes it difficult to achieve high flour protein content. A focus on improving both protein content and protein quality of Oregon cultivars will help establish a foothold in the demanding Asian market.

The objectives of this study were to:

1. Investigate variation and relationships among protein content and composition, kernel characteristics, flour yields, SDS sedimentation volumes, and dough mixing parameters in progeny developed from parent cultivars with contrasting end-use quality.

2. Evaluate changes in progeny quality that result from backcrossing with a lower quality parent.

3. Characterize variation in HMW and LMW-GS composition as related to SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition as measured by the relative proportions of glutenin, gliadin, albumins and globulins.
Wheat Classification and Uses

Wheat is one of the most important food crops grown worldwide, feeding more than one-third of the world’s population. Wheat is nutritious, can be easily stored and plays an important role as a raw material for a diverse range of palatable foods with unique and pleasing textures (Poehlman and Sleper, 1995). Products made from wheat are important sources of carbohydrates, fiber, protein, and other essential nutrients such as iron, calcium, phosphorus, zinc, potassium, magnesium and B vitamins (Halverson and Zeleny, 1988). The endosperm storage proteins (glutenin and gliadin) interact when mixed with water, to form gluten, the viscoelastic matrix of dough (Wrigley et al., 2006). The unique properties of wheat gluten enable the preparation of a range of food products including bread, noodles, cakes, cookies and breakfast cereals. Several species of wheat are cultivated around the world. However, hexaploid or common wheat (Triticum aestivum L. 2n=42; 21”; AABBDD) is by far the most abundant and versatile.

There is no worldwide standard for the commercial classification of wheat. However, distinct market categories are recognized. In the U.S., for example, eight market classes of wheat (durum, hard red spring, hard red winter, soft red winter, hard white, soft white, unclassed, and mixed) are distinguished by the Official U.S. Standards for Grain (USDA-FGIS, 2006). Classes are differentiated by mechanical factors such as grain hardness (hard or soft), growth habit (spring or winter) and bran color (red or white). Wheat quality is further described by grain protein content, test
weight, amount of contamination with material other than the predominant grain, seed soundness, moisture content, and measures of dough processing and product quality (Halverson and Zeleny, 1988).

**Hard White Wheat (HW)**

Hard white wheat was established as a U.S. market class on May 1, 1990. It is defined by the combination of hard endosperm texture and unpigmented seed coat (white bran). Kernel texture, or grain 'hardness', is a primary means of classifying wheat for commerce, and may be its single most important trait in terms of utilization and end-use quality (Morris et al., 2001). Hard wheats require more energy to mill and yield larger flour particles with more starch damage than soft wheats (Symes, 1965). As the amount of starch damage increases, so does the rate of water absorption during dough formation and the enzymatic degradation of starch (Van Der Borght et al., 2005). This in turn impacts the end-product functionality. Hard kernel texture is related to the major effect of the *Ha* gene on chromosome 5DS (Symes, 1965; Campbell et al., 2001) with variations attributed to mutations or deletions in the linked *pin-A* or *pin-B* genes (Morris et al., 2001).

Kernel seed coat color is a highly heritable trait (Cooper and Sorrells, 1984). The color of HW is attributed to recessive alleles at all three major *R* genes on group 3 chromosomes (Sears, 1944; Metzger and Silbaugh, 1970). However, color expression is highly variable due to six minor genes (Freed et al., 1976; Reitan, 1980) and environmental conditions (Wu et al., 1999). Variations in kernel color can be identified by a subjective visual test or quantified using a colorimeter to assess
levels of $L^*$, $a^*$ and $b^*$ as established by the Commission Internationale de l'Eclairage (CIE) (Peterson et al., 2001b).

Hard white wheat has a potentially higher flour extraction rate in milling, since more bran can be included in the flour without darkening it. Consumers often prefer HW because of its lighter color and less bitter taste due to lower phenolic compound content. In addition, reduced amylose content related to null alleles at one or more of the three $Wx$ loci (Nakamura et al., 1992; Graybosch et al., 1998) may improve the suitability of HWs for certain noodle products. Endosperm protein composition is another major factor affecting end-use suitability of wheats. Protein composition can vary widely among wheat classes and cultivars. Newly developed HW cultivars are most similar in the endosperm protein composition and end-use quality attributes to established U.S. hard red wheat cultivars (Pike and MacRitchie, 2004). Accordingly, and contingent on protein content, HW cultivars may be suitable for pan breads, tortillas, or oriental noodles (Lin and Vocke, 2004).

Quality targets for the HW class have evolved to fulfill the need for a dual-purpose, bread and noodle-making wheat. The diverse end-uses of HW present a challenge for plant breeders (Souza et al., 2004). Wheat breeders must address the requirements of both the wheat producers and processors by improving adaptation and yield as well as functionality for many end-products (Ross, 2003).

Wheat Endosperm Components

Starch and protein are the two main endosperm components in wheat. Starch is the major storage component in the endosperm (about 75% of dry weight) and
starch synthesis has a notable influence on grain yield. Wheat endosperm contains 10 to 13% protein (Wrigley et al., 2006). The proteins are traditionally divided into four classes according to their solubility: glutenin, gliadin, albumin, and globulin (Osbourne, 1907). Other minor but important components are lipids and non-starch polysaccharides (mainly arabinoxylans). However, these are of minor importance in the context of the current study and will not be further reviewed.

**Starch**

The water-insoluble granules of starch are the principle carbohydrate store in a wheat seed. There is a bimodal distribution of two types of starch granules in wheat: large, lenticular shaped A-types and small, spherical B-types. Internal molecular organization of the granules gives starch partially crystalline and birefringent properties.

The glucose polymers amylose and amyllopectin are the two major components of starch (Goesaert et al., 2005). The larger, highly branched amyllopectin polymers typically contribute 72-75% of the total weight of the starch. The amyllose polymers are essentially linear and typically contribute 25-35% of the total starch weight. High amyllose (up to 70% amyllose content) and waxy (Wx); (99-100% amyllopectin) mutants have been identified. These mutant types may have the potential to enhance or modify end-product quality (Nakamura et al., 1992; Graybosch et al., 1998).

Starch functionality is attributed to the ratio of amyllose and amyllopectin polymers which affect bread and noodle properties. The eating quality of noodles is
strongly influenced by the pasting and swelling properties of starch (Miskelly and Moss, 1985; Crosbie, 1991), which are primarily starch functionalities influenced by the amylase to amylopectin ratio.

Starch granules swell and gelatinize if heated in the presence of water. On cooling, over time, the glucose polymer chains begin to reassociate, further changing the starch structure. This process, known as retrogradation, contributes to staling and texture changes in bread crumb during storage. Damage to starch granules during milling causes a loss of birefringence, raises water absorption levels, and increases susceptibility to enzymatic hydrolysis (Hoseney, 1994). The level of starch damage varies with the severity of grinding and the hardness of the wheat; soft wheat incurs much less starch damage than hard wheat. In bread-making, a relatively low level of starch damage (although higher than that in soft-wheat flour) is desirable to optimize hydration and promote fermentation through the release of maltose resulting from enzymatic hydrolysis of the damaged granules by endogenous or added amylases. However, excess starch damage may promote more enzymatic hydrolysis than desired, leading to gummy or sticky crumb attributes.

**Glutenin**

Glutenins are polymeric proteins linked by disulfide bonds (Southan and MacRitchie, 1999). Gluten polymers consist of low molecular-weight glutenin subunits (LMW-GS; ~40 kDa) linked by interchain disulphide bonds to high molecular-weight glutenin subunits (HMW-GS; ~90 kDa) (Dupont and Altenbach, 2003).
High Molecular Weight Glutelin Subunits (HMW-GS)

The HMW-GS are encoded by the three loci, *Glu-A1, Glu-B1, and Glu-D1*, located on the long arms of chromosomes 1A, 1B, and 1D, respectively (Payne et al., 1984). Specific alleles have been identified at each of the *Glu-1* loci. At least 104 alleles (McIntosh et al., 1998) of more than 20 HMW-GS at the *Glu-A1, Glu-B1, and Glu-D1* loci have been described (Veraverbeke and Delcour, 2002).

It is easy to distinguish HMW-GS by SDS-PAGE (Payne et al., 1979). An *x*-type and a *y*-type HMW-GS are encoded by two genes at each locus (Payne et al., 1981; Shewry et al., 1992). In general, *x*-type subunits have a higher molecular weight and thus slower electrophoretic mobility than *y*-type subunits (Lafiandra et al., 2000). A numbering system to identify HMW-GS according to electrophoretic mobility and chromosomal location was introduced by Payne and Lawrence in 1983 and has been commonly accepted as the current naming system. Each HMW-GS is identified by its genetic locus (1A, 1B or 1D), type (*x* or *y*), and a number that was originally meant to reflect an order of decreasing molecular weight. HMW-GS with intermediate molecular weights that have since been identified are numbered in order of their discovery (Veraverbeke and Delcour, 2002). HMW-GS nomenclature is commonly abbreviated to just the reference number (i.e. 1Dx5 plus 1Dy10 is often shortened and referred to as 5 +10).

Due to gene silencing in hexaploid wheat, only three to five of the six potential HMW-GS are expressed (Lafiandra et al., 2000). At *Glu-A1*, the *y*-type locus is always silent and the *x*-type locus is sometimes silent. The *y*-type locus at *Glu-B1* also varies in its expression.
Dough strength and baking performance have been related to variation in
HMW-GS. (Payne et al., 1981; Payne et al., 1987; Wrigley and Bietz, 1988; Weegels
et al., 1996; Cornish et al., 2001; Békés, 2001). The Glu-D1 subunits have the
greatest effect on dough properties, followed closely by Glu-A1 alleles. Glu-B1
subunits are least influential (Payne et al., 1981).

Impact of individual HMW bands and band combinations were characterized
by Payne et al. (1984). Bands 1 and 2* are HMW-GS controlled by the alleles at
Glu-A1 locus (Glu-A1a and Glu-A1b, respectively). These two bands have an equal
and positive effect over the null allele (Glu-A1c) (Luo et al., 2001). Several alleles at
the Glu-B1 locus produce intense double bands in SDS-PAGE (17+18, 13+16, and
7+8) in comparison to single or faint bands (7+9, 7+null, and 6+8). Intense double
bands are associated with higher bread-making quality (Singh et al., 1990a). The
superior bread-making properties of hexaploid wheat are largely attributed to alleles
at the Glu-D1 locus; the 5+10 band combination is considered superior to 2+12
(Payne et al., 1984). The effects of the HMW alleles have been shown to be
cumulative (Gupta et al., 1994). However, variation in HMW-GS alone is
insufficient to account for differences in quality; LMW-GS must also be taken into
consideration (Gupta et al., 1994).

Low Molecular Weight Glutenin Subunits (LMW-GS)

Many studies have been done showing the similarities of LMW-GS with both
HMW-GS and gliadins. Early investigations suggested that HMW-gliadin may in
fact be more accurately identified as LMW glutenin and were redefined as such
(Nielsen et al., 1968; Bietz and Wall, 1973). A later study found LMW-GS linked with HMW-gliadin subunits, which are coded for on the short arms of chromosomes 1A, 1B, and 1D (Jackson et al., 1983). Allelic genes at Glu-A3, Glu-B3, and Glu-D3, on 1AS, 1BS, and 1DS, respectively, were found to control LMW-GS banding patterns (Gupta and Shepherd, 1990). Two more genes coding for LMW-GS (Glu-B2 and Glu-B4) were identified by Liu (1995) and Liu and Shepherd (1995), respectively. Evidence has associated LMW-GS with group 6 chromosome short arms as well (Masci et al., 2002).

LMW-GS comprise nearly one-third of the total seed protein and almost 60% of the total glutenins (Bietz and Wall, 1973). Despite their abundance, LMW-GS have not been investigated to the same extent as HMW-GS. Difficulty identifying LMW-GS with one-dimensional SDS-PAGE is one of the main reasons the subunits have not been investigate more thoroughly. A procedure to separate HMW-GS and LMW-GS from albumins and globulins was reported by Gupta and Shepherd (1990) and modified by Singh et al. (1991). The ability to look at gluten subunits in a background free of albumins and globulins has enabled further investigation of LMW-GS. Gupta and Shepherd (1990) detected 20 different LMW-GS band patterns in 222 hexaploid wheat cultivars. This number was much lower than expected on the basis of random association, indicating that the genes controlling the LMW-GS are closely linked (Gupta and Shepherd, 1990).

At this time, there is no commonly accepted nomenclature for identifying LMW-GS. Heterogeneous size and charge characteristics have been used to divide LMW-GS into three types (B, C, and D) (Payne and Corfield, 1979; Gupta and
The most abundant LMW-GS present are the B-type (Masci et al., 2002; Tao and Kasarda, 1989). The C-type typically consists of fewer LMW-GS plus some γ and α-gliadins are also present in this mobility group (Payne and Corfield, 1979; Payne et al., 1985; Kasarda et al., 1988). C-type subunits have greater mobility than the B-type subunits. The least mobile LMW-GS units are classified as D-type (Payne et al., 1985).

Structural differences associated with functionality have been found between the B-type and C and D-types (Kasarda, 1989). The B-type LMW-GS have the ability to form more than one inter-molecular disulfide bond, allowing them to act as polymer chain extenders. On the other hand, C and D-type subunits act as chain terminators, as they only have one cysteine available for the formation of inter-molecular disulfide bonds (D’ovido et al., 2004).

LMW-GS at the *Glu-1* and *Glu-3* loci are associated with dough resistance and extensibility, with particular LMW-GS allele combinations enhancing the parameters more than others (Cornish et al., 2001). Null LMW alleles generally have detrimental effects on dough resistance and extensibility (Benedettelli et al., 1992). However, nearly twice as much LMW-GS is needed to obtain the same dough resistance as compared with HMW-GS (Weiser and Kieffer, 2001). Nonetheless, quality differences have been found associated with differences in the total amount of LMW-GS (Gupta and MacRitchie, 1994). Contradictions are not uncommon among reports comparing effects of LMW alleles. Environment and gene interactions influence relative effects of alleles on end-product quality (Gupta et al., 1994).
Gliadin

Gliadin is the most abundant storage protein in wheat, about 40% of flour protein. These proteins are monomeric, range in size from 30 to 60 kDa and are soluble in 70% alcohol. Four groups of gliadins (α, β, γ, and ω) have been identified based on their electrophoretic mobility (highest molecular weight to lowest molecular weight, respectively) (Gianibelli et al., 2001).

Genetic studies of gliadin proteins have found genes coding for these proteins on the short arms of group 1 (Gli-A1, Gli-B1, and Gli-D1) and group 6 chromosomes (Gli-A2, Gli-B2, and Gli-D2) (Wrigley and Shepherd, 1973). Allelic variants at each Gli locus encode a “block” of linked gliadin polypeptides differing in number, electrophoretic mobility and staining intensity of electrophoretic bands (Sozinov and Poperelya, 1980). Differences in gliadin allele frequencies have been found traced to landraces and ancestral cultivars and can differentiate winter and spring wheats (Metakovsky, et al., 2006).

It has been generally accepted that an increase in the proportion of gliadins is associated with dough flow and extensibility and decreased dough strength (Gianibelli et al., 2001; Peña et al., 2005). However, the affects attributed to gliadins have been questioned with the discovery of tight genetic linkages between gliadins and LMW-GS (Jackson et al., 1983). It is known that gliadins can form intra-molecular disulphide bonds, but no indications of inter-molecular disulphide bonds have been found (D'Ovidio and Masci, 2004). Gliadins do however interact with other molecules in dough formation through their hydrophobic characteristics and hydrogen bonding (Wrigley and Bietz, 1988).
The abundance of Gli alleles and the uniqueness of individual blocks of genes make gliadin polymorphism an efficient tool for wheat genotype identification and differentiation (Uthayakumaran et al., 2006). However, further research is needed to understand the relation between quality and gliadins. Limited knowledge and lack of investigation into gliadins arises from the correlation to weaker doughs that are less tolerant to extended mixing, a minor contribution to viscoelastic properties, and their complex, multigenic loci inheritance (Branlard and Metakovsky, 2006).

**Albumin and Globulin**

Classification of storage proteins describes albumins and globulins as soluble in water and salt solutions, respectively (Osbourne, 1907). Genes for some albumins have been found on 4DL, 4BS, and 3DS (Singh and Skerritt, 2001). Globulin genes have been assigned to chromosomes 4DS, 5DL, 6DS, 7BS, and 7DS (Singh and Skerritt, 2001). These proteins are much higher in lysine, compared to gluten and gliadin fractions. However, albumins and globulins are present in such minor proportions they can not compensate for the overall lack of lysine in the wheat flour (Gianibelli et al., 2001). No significant contributions to dough quality have been attributed to albumins and globulins. Non-gluten proteins may however, act as nutrient reserves for germination, enzymes, structural proteins, or insect and fungal pathogen inhibitors (Dupont and Altenbach, 2003). End-use functionality may be affected by the effects of lipid binding proteins, enzymes and inhibitors (Veraverbeke and Delcour, 2002).
Endosperm Protein Composition and Quality

Endosperm protein composition can be investigated and related to end-product quality based on protein content, the ratio of polymeric (glutenin) to monomeric (gliadin) protein, the ratio of HMW to LMW-GS, and the proportions of \( \alpha \) - and \( \gamma \)-type HMW-GS. The protein content of cereal grains generally ranges from 8 to 16% often varying with environmental influences. Storage protein composition, in terms of the presence or absence of HMW and LMW-GS, is independent of protein content and therefore largely independent of environmental influence (Payne et al., 1984). As milled flour is largely endosperm material (Bass, 1988), flour protein composition is a close reflection of endosperm protein composition. In general, flour protein is composed of 33% gliadin and 16% glutenin (Atwell, 2001). The glutenin portion consists of 10% HMW-GS and 40% LMW-GS (Payne, 1984).

The viscoelastic properties of wheat-flour doughs are imparted by the interaction of glutenin and gliadin. Glutenins confer elasticity whereas gliadins confer extensibility (Payne et al., 1984). Thus the ratio of polymeric to monomeric proteins can be related to the balance of dough strength and extensibility. The proportions of glutenin and gliadin in flour are influenced by genotype and environment, with gliadin most sensitive to environmental influence (Graybosch et al., 1996; Panozzo and Eagles, 2000).

The size of glutenin polymers has been found to be most influential in determining dough strength, in comparison to the glutenin to gliadin ratio (Panozzo and Eagles, 2000). Cultivars with high ratios of HMW to LMW-GS generally possess good bread-making quality (Huebner and Wall, 1976). Variation in flour
quality parameters have been found related to changes in the relative proportions of HMW and LMW alleles (Luo et al., 2001). The potential to improve dough extensibility through use of allelic variation in LMW-GS has been noted in the past (Gupta et al., 1989; Payne et al., 1987). With the ability to form large aggregates, LMW-GS can have a significant impact on dough strength (Gupta et al., 1994). However, the most accurate assessment of LMW-GS qualities needs to be done in conjunction with HMW-GS (Gupta et al., 1994). Manipulation of the ratio of $x$- to $y$-HMW-GS, while maintaining protein content, glutenin to gliadin, and HMW to LMW-GS ratios, also has been shown to change the protein balance in a dough (Butow et al., 2003).

**Predicting End-product Quality**

The term 'quality' is often used to indicate the performance of a cultivar, at a specific protein level, in a test that reflects a specific end product (Bushuk, 1998). End-product evaluation is seldom possible until later generations of a breeding program, due to grain or flour limitations. However, the functional properties of wheat can be predicted from small sample sizes by testing parameters such as protein content and composition (Weegels et al., 1996; Cornish et al., 2001; D'Ovidio and Masci, 2004), kernel characteristics (Bushuk, 1998; Peterson et al., 2001), starch composition (Goesaert, et al., 2005), and dough rheology (Finney and Shogren, 1972; Unbehend et al., 2004).
**Determining Protein Content**

Protein content has an overwhelming influence on end-product quality in wheat. Processors generally establish a minimum protein content to suit their particular end-use needs. Environment is the major determinant of protein concentration; however, variability between wheat cultivars in their ability to accumulate protein is also important.

The total amount of organic nitrogen in flour can be used to determine the amount of protein in the flour. Traditionally, the Kjeldahl method was used to determine nitrogen content of wheat and flour (American Association of Cereal Chemists (AACC) Approved Method 46-10). The basic principle of the procedure is converting protein nitrogen to ammonia, complexing it and titrating it against a standardized sulphuric acid solution. Another AACC approved method (46-30) utilizes the LECO® FP-528 Nitrogen/Protein Determinator. This is a combustion method using high-temperature, pure-oxygen atmospheres to release nitrogen from the protein. Because neither method measures protein directly, the amount of nitrogen measured by these processes is then converted to percent protein by multiplying by a factor of 5.7 for wheat flour (AACC Approved Method 46-19). Near-infrared reflectance spectroscopy can also be used to determine protein content (AACC Approve Method 39-11).
Determining Protein Composition

Glutenin Subunit Identification

Sodium Dodecyl (lauryl) Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) allows fractionation of glutenins based on molecular mass. Proteins are denatured to their primary protein structure and induced with a negative charge by the detergent, SDS. Particles are then migrated through a polyacrylamide gel matrix with an electric current. Smaller proteins will move through the matrix at a more rapid rate than larger proteins. Bands (groups of proteins of the same size) can be visualized by staining the proteins in the gel with a stain such as Coomassie Blue (Neuhoff, et al., 1988).

Procedures have been developed to resolve the HMW-GS and LMW-GS of wheat by SDS-PAGE (Gupta and MacRitchie, 1991; Singh et al., 1991). HMW-GS are more easily extracted and resolved by SDS-PAGE (Payne et al., 1984). Difficulties separating and scoring LMW-GS have required more refined techniques. Extraction procedures eliminating the presence of gliadins, albumins, and globulins have been fundamental to more effectively visualize, score, and characterize LMW-GS (Gupta and MacRitchie, 1991; Singh et al., 1991).

Molecular weight distribution

Molecular weight or size distribution of protein polypeptides and protein aggregates can be examined by Size Exclusion – High Performance Liquid Chromatography (SE-HPLC). The size of gluten proteins and the proportions of polymeric and monomeric proteins are indicators of protein quality (Autran, 1994).
Three main classes of storage proteins can be identified with SE-HPLC: glutenin, gliadin, and albumin-globulin (Larroque et al., 1997). The amount of polymeric protein and the size distribution of these particles have been positively correlated to functional properties of wheat (Gupta et al., 1993).

Proteins are separated based on molecular size using a column which elutes large proteins (polymeric) more rapidly than small proteins (monomeric). This separation allows molecular weight estimates to be determined from the respective elution times of the sorted proteins (Bietz, 1984; 1985). Many computer programs are available to analyze and indicate elution times, absolute and relative proportions of peak areas, and the apparent molecular weight of each peak (Autran, 1994).

Improvements in methods allow for complete extraction of proteins with the use of sonication (Singh et al., 1990b). This results in more reliable prediction correlations between a wheat genotypes and end-use quality (Autran, 1994).

**Kernel Characteristics**

Kernel hardness, weight and diameter are important quality characteristics to consider, as the wheat kernel is the marketed commodity. These characteristics can be indicative aspects of the end-product functionality of a wheat.

Kernel hardness is a highly heritable trait (Baker, 1977), largely independent of changes in protein composition (Luo et al., 2001). The difference between hard and soft wheat kernel texture was found to be due to a single major Ha gene (Symes, 1965; Campbell et al., 2001). Friabilin, a marker protein for grain softness (Ha) consists of two proteins puroindoline a (pinA) and puroindoline b (pinB) which have
been linked to the *Ha* locus. Wildtypes of these proteins are found in greater abundance in soft wheats than hard wheats. Various mutations in the puroindoline proteins result in hard kernel texture and variations of kernel texture within the hard class of wheat (Giroux and Morris, 1998; Morris et al., 2001, Beecher et al., 2002).

Several methods to measure grain hardness have been approved by the American Association of Cereal Chemists (AACC). The hardness of a kernel can be tested by near-infrared absorption (NIR) (AACC Approved Method 39-70A), particle size of milled product (AACC Approved Method 55-30), or the force to crush individual kernels (AACC Approved Method 55-31). Ultimately, hardness affects the amount of starch damaged during milling and consequentially the water absorption capacity of the flour (Mok and Dick, 1991).

Kernel weight and diameter are relatively minor characteristics; however, they are still very useful in predicting milling performance. Size of the grain influences the force required to crush a kernel, therefore kernel size is a contributing factor to hardness (Pomeranz et al., 1990). Kernel morphology, such as length, width, bran thickness, and furrow depth, along with volume and mass of the grain, can also be used as indicators of milling yields (Mabille and Abecassis, 2003). Kernel weight is a function of kernel size and kernel density; large dense kernels usually have a higher ratio of endosperm to non-endosperm components, indicating higher flour yield potential (Halverson and Zeleny, 1988).
**Milling Quality**

Wheat kernels can be separated into fractions of endosperm, bran and germ by milling and sieving. Milling quality can be described as obtaining the highest yield of endosperm material with the least contamination of bran, which is associated with darker flour color and bitter taste.

An estimate of how much flour could be extracted from a given wheat on a commercial mill can be deduced with small scale laboratory milling (Atwell, 2001). Commercial milling yields are often higher due to better separation of particles and flexibility in adjusting the machinery (Atwell, 2001). Because milling yield is in part determined by kernel morphology, estimates of milling yield can also be made from kernel volume/surface ratio calculations (Mabille and Abecassis, 2003). Flour yield values are calculated as a percentage of the flour obtained from the initial weight of cleaned and tempered grain.

**Color**

Color is an important quality character for oriental noodles. Noodle classes differ in color requirements being either white or yellow depending on the absence or presence of alkali salts, respectively. Bright color and minimal darkening during storage are important characteristics in all noodles. Flour whiteness is most frequently associated with good noodle color. Flour, and hence noodle color, is significantly influenced by several factors including protein content, seed coat color, milling extraction, starch damage and PPO enzyme activity (Miskelly, 1994; Hou,
Generally, lower milling extraction rates are associated with brighter and whiter noodle color (Kruger et al., 1994).

The CIE international color measurement system is typically used to evaluate noodle color in the laboratory. Variations in CIE levels of L* (lightness), a* (red-green), and b* (yellow-blue) are assessed, with L* > 91 indicating acceptable noodle whiteness (Hou, 2001). Raw noodle color, especially L*, can vary dramatically among different wheat cultivars (Morris et al., 2000) and environments (Guttieri et al., 2005). Noodle brightness is also inversely related to protein content and to flour-grade color (Miskelly and Moss, 1985).

Polyphenol oxidase (PPO) plays a pivotal role in darkening and discoloration of many wheat products including Asian noodles (Baik et al., 1994; 1995) and bread products (Martin et al., 2005). PPO is found in the aleruone, however it can be passed into flour during milling. This is especially true as flour extraction rates increase (Jimenez and Dubcovsky, 1999). Genes coding for PPO activity have been located on chromosome 2D of wheat (Jimenez and Dubcovsky, 1999; Anderson and Morris 2001). Screening for noodle discoloration potential of HW is achievable with enzyme assays using various substrates such as phenol, L-tyrosine, catechol, methyl catechol, or 3,4 dihydroxyphenyalanine (L-DOPA). A non-destructive enzyme assay described by Anderson and Morris (2001) can be done in as little as 30 minutes with only one to five seeds. Using L-DOPA as a substrate, seed viability is minimally affected. The assay provides a robust and efficient method to evaluate germplasm and cultivars for PPO activity (Anderson and Morris, 2001).
Dough Rheology

Rheology is the study of the deformation and flow of matter. In dough, rheological properties are mostly defined by the gluten proteins and the interactions among them. The physical properties of doughs can be directly determined using instruments, such as the Farinograph, Extensograph, Alveograph, and Mixograph to measure the physical response of doughs to external forces or dough properties can be predicted by sedimentation tests (Cornish et al., 2006).

Sedimentation Volume

Sedimentation volume tests, such as sodium dodecyl sulfate (SDS) or Zeleny can be used to predict gluten quality and loaf volume potential (Cornish et al., 2006). SDS sedimentation volume testing is often preferred as a measure of protein quality due to the higher average heritability and relative simplicity (O’Brien and Ronalds, 1987). SDS sedimentation volume assesses gluten strength by swelling gluten proteins from ground wheat or flour in the presence of lactic acid and measuring the volume of sediment (AACC Approved Method 56-60). The microsedimentation procedure described by Dick and Quick (1983) is fast and reproducible requiring a maximum of 1 g of ground sample and relatively inexpensive and unsophisticated equipment. This is especially advantageous for early generation screening.

Physical Dough Quality Instrumentation

The Farinograph is used to determine the optimum water absorption capacity of flour and evaluate the development and stability of doughs during mixing (AACC
Approved Method 54-21). The mixing action is provided by two sigma-type blades rotating in opposing directions. The Extensigraph and Alveograph record extensibility and resistance to extension of doughs as a force-time curve for a piece of dough stretched until it breaks (AACC Approved Methods 54-10 and 54-30, respectively). The Farinograph, Extensigraph, and Alveograph require 50 to 300 g of flour, which is a disadvantage for use in early generation testing for wheat breeding programs. However, investigations into the use of micro-extensigraph methods have shown promise in early generation wheat quality screening (Grausgruber et al., 2002).

The Mixograph is a recording dough mixer that can evaluate 2 to 35 g samples (AACC Approved Method 54-40A). A mixogram curve is obtained by recording the force exerted on mixing pins by a dough at a given water absorption level (AACC Approved Method 54-50). Data can be collected from curves by hand or with the assistance of MIXSMART® computer software (Walker and Walker, 1992). In either way of interpreting data, a curve is drawn relative to the midpoint of the band and peak, slope, and width parameters are measured relative to this line (Atwell, 2001). An example of a mixogram is presented in the Materials and Methods section.

Height of the curve at the peak is related to water absorption and protein content. The peak height increases as protein content increases (Atwell, 2001). The amount of time it takes for a sample to reach its peak (mix peak time) is also indicative of dough-strength and end-product functionality (Finney and Shogren, 1972). This is the point in dough mixing where gas retention in a leavened dough
and ultimately bread quality will be optimum (Atwell, 2001). Mix peak time is influenced by protein content, protein quality, and oxidation ability of the flour (Finney and Yamazaki, 1967). Optimal mixing times vary for different products. Generally a mix peak time of three to four minutes is desirable. As mixing time increases, generally dough extensibility decreases and dough stability, elasticity, and mixing tolerance increase (Atwell, 2001). Over time, as mechanical degradation increases and dough mobility increases, the mix curve slopes downward and diminishes to varying degrees, indicating a breakdown of the gluten protein (Finney and Shogren, 1972). This can be translated into a measure of mixing tolerance. Mixing tolerance is often scored against standard curves on a scale of 0 to 7 or as width of the curve two minutes past the peak, with higher scores indicating greater tolerance to overmixing (Baenziger et al., 2001).

Relationships of Environment and Genetic Background to Protein Composition and End-Product Functionality

Development of HW cultivars with stable end-product functionality when grown in diverse environments is essential to the establishing markets for U.S. HW. Understanding environmental influences is important to improving end-product functionality and stability.

Genotype is a major source of variation in protein composition, in terms of genes that control the presence or absence of protein subunits. However, variation in subunit composition can not explain the influence of environment or interactions
between genotypes and environments, which are also important sources of variation (Graybosch et al., 1996).

It is well established that end-use quality is associated with grain protein content, which can be significantly influenced by N-fertilization and environment (Graybosch et al., 1996; Wieser and Seilmeier, 1998; Panozzo and Eagles, 2000). However, variation in protein concentration alone does not adequately explain end-use quality characteristics (Peterson et al., 1992). Cultivar reactions to temperature (Dupont and Altenbach, 2003), water stress (Guttieri et al., 2000), and micro-nutrient deficiencies (MacRitchie and Gupta, 1993) may also impact protein development and total protein content in wheat.

Environmental stresses may cause variation in the ratios of gliadin to glutenin and HMW-GS to LMW-GS (Gupta et al., 1992; Wieser and Seilmeier, 1998; Veraverbeke and Delcour, 2002). Protein content, gliadin, and non-gluten proteins have been found to be most sensitive to variation in environmental conditions (Graybosch et al., 1996). Perrotta et al. found no differences in HMW-GS genes expression under temperature stress (1998). Gupta et al. found relative effects of HMW and LMW-GS on dough properties were consistent over environments and at varying protein levels, indicating that dough strength can be improved without losing yield potential by increasing protein levels (1994). However, Altenbach et al. (2002) showed that accumulation of transcripts for major gluten protein genes was slightly earlier with high temperatures at grain fill. Lafandria et al. (1999) reported high temperatures at grain fill reduced the molecular weight distribution of gluten by interrupting disulfide bond formation resulting in weaker-than-expected doughs.
MacRitchie and Gupta (1993) observed increases in the ratio of HMW and LMW-GS as sulfur content decreased.

Environmental influences and the subsequent biochemical changes they impart can impact bread and noodle quality. Loaf volume and SDS sedimentation volume have consistently shown a curvilinear response to heat stress where no change in protein content was observed, indicating a change in protein composition (Peterson et al., 1998). Nitrogen fertilization rates were correlated to increases in grain protein, water absorption, loaf volume, and mix peak height and time (Guttieri et al., 2005). Increased protein concentration and loaf volume were found to indicate improved noodle texture (Souza, 2004). Noodle color was found to be affected by genotypic, environmental and genotypic x environmental effects, but low environment and genotype x environment effects were found for noodle texture (Graybosch et al., 2004). Reduced flour extraction rates and increased PPO activity, yellowness of noodles, and flour pasting viscosity were all related to late-season moisture stress (Guttieri et al., 2005). Guttieri et al. (2000), found traits such as mix peak time and loaf volume/protein ratios are not impacted by genotype x environment interaction effects and can therefore be selected effectively in early generations. However, traits such as grain protein, flour yield, and loaf volume were subject to genotype x environment interaction and require multi-environment evaluations for selection.

Application of genomic and proteomic studies creates potential to better understand of the role of environment in determining productivity and quality of wheat (Altenbach et al., 2003). These studies may resolve complex patterns of gene
expression during grain development, pinpoint key regulatory processes that are influenced by the environment, and reveal the molecular basis for environmental variation on flour composition and quality (Dupont and Altenback, 2003).

Environment and genotype both determine protein content and composition of a cultivar. Interactions between genotype and environment need to be considered in the development of a HW cultivar with good end-product functionality. Genetic potential to improve consistency and stability of quality traits in hard red wheat cultivars across production regions has been indicated by Peterson et al. (1992). Given the similarities between HRW and HW reported by Pike and MacRitchie (2004), there is similar opportunity in HW development.
Materials and Methods

Plant Materials

Four diverse parental cultivars were used to develop three wheat populations (Table 1). OR943576 and OR850513-8 are experimental lines from the Oregon State University Wheat Breeding Program. OR943576 has the pedigree MRS/Ci114482/M/YHY/3/RONDEVOUS. OR850513-8 has a different genetic background with the pedigree RBS/ANZA/3/KVZ/HYS//YMH/TOLB/4/BOW S. Both lines have shown promising agronomic performance but have weak dough mixing properties, soft noodle texture, and protein composition that makes them unsuitable for bread and noodle making. Two cultivars from Nebraska, N97S277 and N96L1226, were used to introduce superior protein quality and baking characteristics. The hard white wheat (HW) N97S277 is a sib of the Nebraska cultivar ‘Antelope,’ derived from the cross ‘Pronghorn’/’Arlin’ (Reg. no. CV-968, PI 633910, Graybosch et al., 2005). The hard red wheat N96L1226 is a sib of ‘Wesley,’ with the pedigree ‘Plainsman V/Odesskaya 51’/’Colt/Cody’ (Reg. no. CV-890, PI 605742, Peterson et al., 2001). Both lines have medium to strong gluten characteristics and good baking quality. The lines were not previously evaluated for noodle quality. However, both have relatively high polyphenol oxidase (PPO) enzyme activity levels, which generally result in unacceptable noodle color.

Single cross and backcross combinations were made among the four parent cultivars (Table 1). The adapted Oregon parent, OR943576, was used as the recurrent parent to generate backcross progeny in Populations 1 and 2 (Table 1). Populations were
Table 1. Parent cross combinations for Populations 1, 2, and 3.

<table>
<thead>
<tr>
<th>Population</th>
<th># F3 derived lines</th>
<th>Pedigree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>OR943576* / N97S277‡</td>
</tr>
<tr>
<td>1</td>
<td>114</td>
<td>OR943576 // OR943576 / N97S277</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>N96L1226§ / OR943576</td>
</tr>
<tr>
<td>2</td>
<td>96</td>
<td>N96L1226 / OR943576 // OR943576</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>OR850513-8† / N97S277</td>
</tr>
</tbody>
</table>

*OR943576 = MRS/CI14482//YMII/HYS/3/RONDEVOUS
†OR850513-8 = RBS/ANZA/3/KVZ/HYS//YMII/TOB/4/BOW S
‡N97S277 = ‘Pronghorn’ / ‘Arlin’
§N96L1226 = ‘Plainsman V/Odesskaya 51’//’Colt/Cody’
planted as F₂ or BCF₂ bulks in the field during the fall of 2001. At harvest, individual heads were snapped and threshed. Harvested seed was visually screened for seed color and heads with red seed were discarded. Single seeds were planted in conetainers (4 x 20 cm) in the fall of 2002 and vernalized for 6 weeks at 5 °C in a growth chamber. Conetainers were then transferred to the greenhouse and maintained on 16 hr light cycles at 18 °C. Environmental stress was minimized by providing adequate moisture and nutrients. Pests were controlled on an as-needed basis. In all, 1845 plants were generated. Seed of each plant was screened and those with high PPO activity were eliminated.

Up to twelve seeds from 891 F₃-derived lines were planted in hydrated 1.5” Jiffy® pots. Problems with seed dormancy became apparent after two weeks at 8 °C in growth chambers. Pots were transferred to a 16 °C greenhouse and treated with 100 μM gibberellic acid (GA₃). Remnant seed for lines that were not recovered were germinated in Petri dishes on germination paper soaked with 500 ppm GA₃ at 10 °C. Once germinated, seedlings were transferred to 1.5” Jiffy® pots and vernalized at 8 °C for four weeks before moving to the greenhouse. Plants were transferred to one-gallon pots and the greenhouse was maintained in an environment conducive to growing winter wheat, treating pests as necessary. Four hundred seventy-eight F₃-derived lines were harvested to provide F₅ seed for field testing.

**Field Design**

Field trials were planted at Hyslop Farm in Corvallis, OR on October 17, 2003. The soil type at Hyslop corresponds to the Woodburn Series (fine-silty,
mixed, superactive, Aquultic Argixerolls). F₃ lines with at least 15 g of seed were sown in 2-row plots on 30.5 cm spacing, 3 m long, with a second replication planted when seed was available. After an initial disease response for stripe rust was recorded on June 9, 2004, disease was controlled with a rate of 4 oz acre⁻¹ Tilt® (Propiconazole) applied on June 14, 2004. Prior to planting 100 lbs Ac⁻¹ nitrogen fertilizer (48-0-0) was incorporated into the soil. Plots were top-dressed 50 lb Ac⁻¹ with ammonium sulfate (33-0-0-12) in the spring with another 100 lb Ac⁻¹ 48-0-0 broadcast prior to grain fill in an effort to enhance grain protein content.

Parental cultivars and two soft white wheat cultivars, Stephens and Eltan, were used as checks. Four replications of each check cultivar were included in each population. Populations 1 and 2 included both single cross and backcross individuals. Population 3 included only single cross individuals. For Population 1, 84 of the lines were planted in two replicates; the other 49 lines were planted with a single replicate. Population 2 had 79 lines planted in two replicates, 60 lines were planted with a single replicate. Population 3 had 38 lines planted in two replicates, 50 lines were planted with a single replicate.

Agronomic Evaluation

Agronomic data were collected on plant height, heading date, lodging, grain yield, and disease response. Height was measured in centimeters, from the ground to the top of the spike, excluding awns. Heading date was determined as days from January 1, 2004, when at least 50% of spikes in the plot were out of the boot.
Lodging scores were taken just prior to harvest and expressed as a percentage of the total plot lodged. Yield was measured in g plot\(^{-1}\) and converted to kg Ha\(^{-1}\).

Reaction to stripe rust (\textit{Puccinia striiformis} Westend. \textit{f. sp tritici. Erikson (Pst)}) and leaf rust (\textit{Puccinia triticina}) were recorded as percentage of leaf area infected and response type: resistant (R), moderately resistant (MR), moderately susceptible (MS), or susceptible (S). A total of 20 lines were eliminated from the study based on susceptibility to stripe rust; 13 from Population 1, 1 from Population 2, and 6 from Population 3. Lines which exhibited a high level of physiological leaf spot as well as lines with intermediate to low grain hardness scores also were discarded before testing end-product quality or protein composition. Ultimately, Population 1 had 94 lines total; 64 lines with two replicates, 30 lines with one replicate. Population 2 had 86 lines total; 49 lines with two replicates, 37 lines with one replicate. Population 3 had 50 lines total; 20 lines with two replicates, 30 lines with one replicate.

**Whole Seed Evaluation**

Polyphenol oxidase activity was determined using a L-DOPA colorimetric procedure (Approved Method AACC 22-85, Anderson and Morris, 2001). Each sample was measure in duplicate using five kernels per assay. In Populations 1 and 3 experimental lines with PPO activity greater than 0.50 A\(_{492}\) were eliminated from the study. To maintain adequate population size in Population 2, all lines were included regardless of PPO activity.
Kernel characteristics were assessed using a Perten Instruments Single Kernel Classification System (SKCS) 4100 (Springfield, IL). Hardness (Approved Method AACC 55-31), weight (mg) and diameter (mm) were determined for 300 kernels per sample.

**Milling**

Grain samples were milled into flour using a modified process on a Brabender Quadromat Sr. laboratory mill. The modified process involved transfers of milled samples to a Tyler Ro-tap Sieve Shaker RX-29 (W.S. Tyler Company, USA) rather than the conventional sieve box fixed to the mill. Milling independent of the attached sieving apparatus permitted the use of less sample and measurement of all milling products. Each 100 g sample was fed into the break mill at a rate of \( \sim 100 \text{ g min}^{-1} \). Material ground through the break mill was then sieved for 4 min using 864, 234, and 140 \( \mu \text{m} \) mesh-screen sieve pans. Bran retained above the 864 \( \mu \text{m} \) sieve pan was weighed and discarded. Break flour passed through the three sieves into the bottom pan; it was weighed and retained for blending. Overs, or residue, from the 234 and 140 \( \mu \text{m} \) mesh sieves was fed into the reduction mill via a magnetic feeder pan at a constant rate of \( \sim 30 \text{ g min}^{-1} \). Stock from the first reduction milling was then sieved for 2 min using the same 864, 234, and 140 \( \mu \text{m} \) mesh-screen pans. Overs from the 864 and 234 \( \mu \text{m} \) mesh sieves (bran) were weighed and discarded. Flour sieved through to the bottom pan was weighed and combined with break flour. Overs from the 140 \( \mu \text{m} \) mesh sieve were fed into the reduction mill for
a second milling, again using the magnetic feeder at a constant rate 30 g min\(^{-1}\).

Second reduction milling stock was sieved for 2 min using the 864, 234, and 140 μm mesh screen sieves. Overs in the three screen pans (shorts) were weighed and discarded. Flour from the bottom pan was weighed and blended with the other flour obtained from the sample to create the laboratory equivalent of straight-grade flour. The mill and sieve pans were cleaned with an air hose before milling the next sample. The total flour yield and break flour yield were calculated as a percentage of total milling products recovered.

**Flour Analyses**

A LECO® FP-528 Nitrogen/Protein Determinator was used to determine protein content of flour samples by combustion (AACC Approved Method 46-30). Approximately 100 mg of flour from each sample was combusted in a 250 mg Quick Cap (LECO®). Five ETDA standards were run intermittently during sample runs. The percentage of crude protein was calculated by multiplying the percentage of N detected by combustion by a factor of 5.70 (AACC Approved Method 46-19).

To determine relative gluten strength, a sodium dodecyl sulfate-microsedimentation test (SDS-MST), as described by Dick and Quick (1983) was used. Two measurements of each sample were run and their results averaged. Mixograms were generated using a 10 g mixograph (National Manufacturing Co., Lincoln NE) and MIXSMART® software (Walker and Walker, 1992). Parent cultivar N97S277, was used as a reference and to determine optimum water
absorption. For each sample, 10 g of flour, 6.2 mL of water, and a spring setting of 10 was used. Samples were run for up to 8 minutes each. Field replicates were compared to ensure reproducibility. Mixing bowls were cleaned and dried between samples to maintain moisture and temperature among samples. Measurements collected from mixogram curves included: time to peak (mix peak time), as a measure of optimal dough development, mixing stability as determined by the slope of the curve after the peak, and mixing tolerance, measured as the width of the curve at six minutes after initiation of mixing (Figure 1).

Protein Composition Analyses

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and size exclusion high performance liquid chromatography (SE-HPLC) were used to determine the protein compositions and protein molecular weight distributions of lines in the three populations.

For SDS-PAGE, both HMW and LMW proteins were extracted from flour samples using methods described by Singh et al. (1991). Slight modifications were made to the procedure to suit equipment available and increase extraction of proteins. Centrifuge time was increased to 5 min in a Brinkman 5413 centrifuge following extraction of gliadins and protein alkylation. Drying after extraction of gliadins was accomplished by placing tubes upside down on Kimwipes® for a minimum of 1 hr instead of using aspiration. The amount of dithioreitol was
Figure 1. Example mixogram and respective measures of mixing properties. A. Mix Time, peak of mix curve. B. Mixing Stability, slope of curve after peak. C. Mixing Tolerance, % of full curve after 6 min of mixing.
increased to 1.5% (w/v) for the extraction of glutenin and the amount of 4-vinylpyridine was increased to 2% (v/v) for protein alkylation.

Protein electrophoresis was conducted on 9.5-14.5% gradient polyacrylamide stacking gels as described in Gupta and McRitchie (1991) for 18 hours at 10 mA gel cooling to 20 °C. Gels were stained with Coomassie Blue G-240 using the Neuhoff (1988) protocol. Banding patterns for HMW-GS were scored on a presence or absence basis similar to Gupta and Shepard (1990). Parental cultivars were scored for five B-type LMW-GS, referred to as a, b, c, d, and e (Figure 2) that were found to be polymorphic. Type-C LMW-GS bands were also polymorphic; however, inadequate resolution prevented accurate scoring of these bands. Images were captured with a digital camera by placing stained gels over a light box.

Relative quantities of polymeric, monomeric, and albumin and globulin proteins were determined by SE-HPLC. Stock solutions of phosphate extraction buffer were prepared by adding 10 g of SDS and 14.196 g of sodium phosphate dibasic anhydrous in 985 mL purified water and adjusting the pH to 6.9. The solution was then brought up to a volume of 1 L. Proteins were extracted from 160 mg of flour. Flour was dispersed in 20 mL of phosphate extraction buffer in a 50 mL tube. Samples were then sonicated for 180 s at 5 W. After sonication samples were spun at 18000 rpm in a Sorvall RC-5B refrigerated high-speed centrifuge for 30 min at room temperature. Supernant was filtered at 0.45 μm with a syringe into the injection tube. Tubes were incubated for two minutes at 80 °C in a water bath.
Figure 2. SDS-PAGE banding patterns of endosperm proteins extracted from parent cultivars, adapted checks, and population individuals.
HPLC was performed as described by Ohm et al., 2006. Extracted samples were loaded and SE-HPLC was performed on a Waters 2695 separations module (Waters, Milford, MA) for 30 min with a BIOSEP SEC S4000 size-exclusion column (600 x 7.5 mm, Phenomenex, Torrance, CA) and a guard column (75 x 7.5 mm). A 20 μL sample was injected and eluted using 50% Acetonitrile in water made up to 0.1% with Trifluoroacetic acid (TFA), as the running buffer at a rate of 1 mL min⁻¹. Eluents were detected at 214 nm using a Waters 2996 photodiode array detector. Transformation of SE-HPLC UV absorbance data was performed as described by Ohm et al. (2006). Data was then integrated into peak areas based on elution times (Figure 3).

Three peaks were identified in each chromatogram (Figure 3) as a proportion of total area under the chromatogram curve. Peak 1 was integrated from 11 to 18.75 min. Peak 2 was calculated from 18.75 to 21.0 min. Peak 3 was calculated from 21.0 to 25 min. Similar to Larroque et al. (1997): Peak 1 consisted mainly of HMW and LMW glutenin with some overlap between LMW-GS and HMW-gliadins; Peak 2 consisted of monomeric proteins, mainly gliadins; Peak 3 consists of monomeric albumins and globulins. For the purposes of this study Peak 1 will be referred to as polymeric protein, Peak 2 will be referred to as monomeric protein, and Peak 3 will be referred to as albumin and globulin.
Figure 3. SE-HPLC separation of proteins fractionated into three peak areas based on elution time. Peak 1 = Polymeric Proteins; Peak 2 = Monomeric Proteins; Peak 3 = Albumin and Globulins.
Statistical Analyses

Data were analyzed using the PROC GLM and LSmeans procedures in SAS® software (SAS Institute, 2004). Analyses were done within each population as well as between population means. The single cross and backcross progeny in Population 1 and Population 2 were compared within each population. Single cross and backcross means within each population and between all three populations were also compared. Distributions of each parameter tested were examined by population.

Paired comparisons (t-tests at p = 0.05) were made between progeny groups defined by HMW and LMW-GS banding patterns. Laboratory results for lines with two field replicates were averaged for analyses. Contrasts were calculated only for HMW and LMW-GS groups represented by at least five individuals. Contrasts among LMW-GS bands were also made within each major HMW-GS class.
Results

Parent Cultivars

Parental cultivars were genetically diverse based on pedigree (Table 1). Compared with the Pacific Northwest (PNW) cultivars, the Nebraska parents N97S277 and N96L1226 were found to be relatively well adapted to the 2003-04 production conditions based on yield, maturity, plant height, and straw strength. Although the field study was not designed to precisely measure grain yield, it was noted that grain yields of the Nebraska cultivars were comparable to those of the Oregon parents and check cultivars (Table 2). N97S277 was the earliest maturing cultivar with 135 days to heading, followed by OR850513-8 (136 days), N96L1226 (138 days) and OR943576 (139 days). These values were comparable to the range established by adapted soft wheat cultivars Stephens and Eltan, at 136 and 147 days to heading, respectively. The Nebraska cultivars reached physiologic maturity within four days of Stephens. Plant heights of N97S277 and N96L1226 were 4 cm taller than Stephens and 3 cm shorter than Eltan, similar to OR850513-8. There was no significant lodging in the trials.

Stripe and leaf rust were severe in 2004. The parents showed moderate to high levels of resistance to stripe rust. Eltan and N96L1226 were moderately resistant with leaf infection ranging from 10 to 20%. None of the other cultivars showed any evidence of infection. OR943576, OR850513-8 and N97S277 were more susceptible to leaf rust than N96L1226. Infection ranged from 40 to 60% on
Table 2. Mean grain yield, kernel weight, kernel diameter, kernel hardness, and PPO enzyme activity (PPO) for parental and check cultivars

<table>
<thead>
<tr>
<th>Line</th>
<th>n</th>
<th>Yield</th>
<th>Kernel Weight</th>
<th>Kernel Diameter</th>
<th>Kernel Hardness</th>
<th>Grain PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>kg Ha⁻¹</td>
<td>mg</td>
<td>mm</td>
<td>HI</td>
<td>A₄₉₂</td>
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<tr>
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<td>12</td>
<td>7062a</td>
<td>41.36bcd</td>
<td>2.78c</td>
<td>68.56b</td>
<td>0.36b</td>
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<td>6893a</td>
<td>38.23d</td>
<td>2.71d</td>
<td>76.95a</td>
<td>0.28bcd</td>
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<td>12</td>
<td>7042a</td>
<td>39.38d</td>
<td>2.66d</td>
<td>68.31b</td>
<td>0.61a</td>
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<td>3.08a</td>
<td>36.68c</td>
<td>0.35bc</td>
</tr>
<tr>
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<td>6741a</td>
<td>39.74cd</td>
<td>2.60e</td>
<td>32.65d</td>
<td>0.26c</td>
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</tbody>
</table>

* Means with the same letter in the same column are not significantly different at p ≤ 0.05
OR943576 and OR850513-8 and 20 to 60% on N97S277. N96L1226 was mostly resistant with only 10% of the leaf area affected.

As expected, the parents exhibited a wide range of diversity in quality characteristics. Polyphenol oxidase (PPO) activity ranged from 0.28 to 0.70 $A_{492}$, with both Oregon parents averaging 0.36 $A_{492}$ or less. N96L1226, a red-seeded cultivar, had PPO levels only slightly higher than that of N97S277, a hard white cultivar. Grain hardness indexes (HI) ranged from 67.45 (N96L1226) to 76.95 (OR850513-8), which is considered satisfactory for a hard wheat market class. Single kernel weight for parents ranged from a low of 38.23 mg for OR850513-8 up to 42.32 mg for N96L1226. This was lower than for Stephens, which is known for its large grain size, but comparable to Eltan. Kernel diameters of parents were similar to or larger than Eltan, but smaller than Stephens, ranging from 2.66 mm to 2.87 mm.

Total flour yield of hard wheats ranged from 68.0 – 71.5%, with the largest difference between OR943576 and N97S277 (Table 3). All parents had higher flour yields than the soft wheat check cultivars, which were under 65%. High break flour yields suggest OR943576 and N96L1226 had relatively better milling performance as compared with N97S277 and OR850513-8.

OR943576 had significantly lower average flour protein although differences among parental cultivars were less than 7.0 g kg$^{-1}$. SDS sedimentation volumes, as a measure of protein quality, were different between the Oregon and Nebraska cultivars and between the two Oregon cultivars: N96L1226 = N97S277 > OR850513-8 > OR943576. Similar tendencies were observed in measurements from
Table 3. Mean flour yield, total flour yield, protein content, SDS sedimentation volume (SDS), mix peak time, mixing stability, and mixing tolerance for parental and check cultivars.

<table>
<thead>
<tr>
<th>Line</th>
<th>Break Flour Yield</th>
<th>Total Flour Yield</th>
<th>Flour Protein</th>
<th>SDS</th>
<th>Mix Peak Time</th>
<th>Mixing Stability</th>
<th>Mixing Tolerance</th>
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</thead>
<tbody>
<tr>
<td>OR943576</td>
<td>12</td>
<td>32.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.6&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>-4.85&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>OR850513-8</td>
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<td>75.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>-7.48&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
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<td>12</td>
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<td>68.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>-4.88&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>69.1&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>-3.45&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
</table>

* Means with the same letter in the same column are not significantly different at p ≤ 0.05
the mixograph. The Nebraska parents had longer mix peak times, N97S277 (4.9 min) and N96L1226 (5.17 min) compared to OR943576 (3.6 min) and OR850513-8 (2.4 min). They also had superior mixing stability (measured as slope of the curve after the peak) and mixing tolerance (measured as width of the band after the peak).

Protein composition varied significantly among all parent cultivars as measured by SE-HPLC (Table 4). The relative proportion of polymeric proteins was significantly greater in the Oregon parents than the Nebraska parents. Monomeric protein was proportionally higher in the Nebraska cultivars than the Oregon cultivars. Proportion of albumin and globulin were similar among parents with the exception of N97S277, which was significantly lower.

Agronomic Performance and End-Product Functionality of Populations and Progeny

Combining ability of the parent cultivars were examined by comparing population means. Differences between Population 1 and Population 2 can be associated with relative contribution of the Nebraska parents; whereas contrasts between Population 1 and Population 3 are associated with contributions of the Oregon parents.

Several experimental lines were eliminated from the study due to their extreme susceptibility to stripe rust. Leaf rust is generally not a problem in the Willamette Valley. In 2004, however, conditions were such that leaf rust was present on all plants, ranging in severity from 10 to 60% of the leaf area. No plants were removed from the populations based solely on their leaf rust susceptibility.
Table 4. Relative proportions of polymeric protein, monomeric protein, and albumin and globulin as determined by SE-HPLC for parent and check cultivars.

<table>
<thead>
<tr>
<th>Line</th>
<th>n</th>
<th>Polymeric Protein</th>
<th>Monomeric Protein</th>
<th>Albumin and Globulin</th>
<th>% of total</th>
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<tbody>
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<tr>
<td>Eltan</td>
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<td>39.78&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>40.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

* Means with the same letter in the same column are not significantly different at p ≤ 0.05
Leaf rust ratings were not significantly correlated to any change in yield, single kernel characteristics or SDS sedimentation volumes.

Grain yields for the three populations were significantly different averaging 7239.28, 6897.63, and 6845.95 kg Ha\(^{-1}\), respectively. Average heading date for Population 1 was 138 days, Population 2 was 139 days and Population 3 was 136 days; as compared with Stephens at 136 days. Plant height of populations ranged from 105 to 109 cm. Population means for these traits were similar to the midpoint of the parents for each population. PPO activity was higher in Population 2 than Population 1 or Population 3 (Figure 4). Higher PPO activity in progeny from the N96L1226, red-seeded parent, was not unexpected, even though all progeny in the study were white seeded. No differences in mean grain hardness were found among the populations; likely due to the removal of low and intermediate kernel hardness progeny. N97S277 had lower average kernel weights and diameters than N96L1226, which likely contributed to the lower average kernel weights and diameters of Population 1. Similarly, lower kernel weights of Population 3 as compared to Population 1 resulted from the lower kernel weight of the OR850513-8 as compared to OR943576. Significant differences in the mean kernel diameter of the Oregon parent cultivars were not observed between Population 1 and 3.

Total flour and break flour yields were significantly lower in Population 1 than Population 2, similar to that observed between the Nebraska parents (Figure 5). Mean flour yield of Population 1 was 3.45% lower than the OR943576 parent, similar to N97S277. Mean flour yield of Population 2 was greater than the mean of either parent. There were significant differences in milling yields between
Figure 4. Means and ranges of grain yield, kernel weight, kernel diameter, kernel hardness, and grain PPO activity for Populations 1, 2, and 3.

- **Grain Yield**
- **Kernel Weight**
- **Kernel Diameter**
- **Kernel Hardness**
- **Grain PPO Activity**

Legend:
- **Population 1**
- **Population 2**
- **Population 3**
- OR943576
- OR850513-8
- N97S277
- N96L1226
Figure 5. Means and ranges of break flour yield, total flour yield, and flour protein content for Populations 1, 2, and 3.
Populations 1 and 3, but there were no obvious relationships with differences between the Oregon parents (Table 3). Milling yield is a highly complex trait and may be related to a number of kernel characteristics, including kernel hardness, weight, diameter, and overall kernel shape (Mabille and Abecassis, 2003).

Mean flour protein levels of each population were significantly ($p \leq 0.05$) different: Population 3 $>$ Population 2 $>$ Population 1 (Figure 5). Protein contents for each of the populations were similar in rank, but slightly lower than respective parental midpoints (Table 3). Populations also differed in mean SDS sedimentation volume (Figure 6). The means of Population 1 and 2 were slightly less than the respective parental midpoints, reflecting the influence of the recurrent parent (OR943576) and the greater number of backcross individuals. The mean of Population 3 was nearly the same as the N97S277 parent, greater than the midpoint of the parents.

Although mix peak time, mixing stability, and mixing tolerance of N97S277 and N96L1226 were not different (Table 3), Population 1 had a slightly longer mix peak time and better mixing tolerance than Population 2 (Figure 6). OR943576 had less mixing tolerance than OR850513-8 (Table 3), which translated to lower average mixing tolerance of progeny from Population 1 as compared to Population 3.

Populations 1 and 2 differed in relative proportions of polymeric, monomeric, and albumin and globulin fractions (Figure 7). Lower proportions of albumins and globulins in Population 1 compared with Population 2 were similar to the difference observed between N97S277 as compared with N96L1226. However, Population 1 also had lower levels of monomeric proteins while its parent, N97S277, had
Figure 6. Means and ranges of SDS sedimentation volume, mix peak time, mixing stability, and mixing tolerance for Populations 1, 2, and 3.
Figure 7. Means and ranges of relative proportions of polymeric protein, monomeric protein, and albumins and globulins for Populations 1, 2, and 3.
relatively higher levels as compared with N96L1226. Differences in polymeric and monomeric protein proportions between Populations 1 and 3 (Figure 7) were consistent with differences in OR943576 and OR850513-8 parents (Table 4). The relative proportion of polymeric proteins is higher in Population 1 and its parent, OR943576. The relative proportion of monomeric proteins was higher in both Population 3 and its parent, OR850513-8.

**Population 1**

Parent cultivars for Population 1, OR943576 and N97S277, differed for all traits except grain yield, grain hardness, and mixing stability. Variation was found among progeny for grain yield, PPO activity, kernel hardness, weight and diameter, flour yields, SDS sedimentation volumes, mix peak time, mixing tolerance, and protein composition. Only protein content and mixing stability did not differ significantly among progeny within the population.

Considerable variation in grain PPO activity was found within Population 1, in spite of an initial screening to remove progeny with high enzyme activity (Figure 4). The average PPO score for the population was significantly less than the midpoint of the two parents. However, progeny with enzyme activity levels above and below the parents were found.

Progeny varied for single kernel characteristics (Figure 5) and flour yields (Figure 6), with averages near the midpoint of the parents. Flour protein content was normally distributed around the population average, ranging from 6.83 to 9.44%.
The average flour protein content in Population 1 was 7.96%, less than either OR943576 (8.27%) or N97S277 (8.82%).

There was a wide range in SDS sedimentation volumes among progeny, greater than that for the parents (Figure 6). The mean of the population was less than the midpoint of the parents. Distributions of SDS sedimentation volumes suggest three main groups of progeny; two generally correspond with parental SDS sedimentation values, the third group was intermediate (Figure 8).

Significant variability was observed in mixing characteristics, ranging from less than OR943576 to greater than N97S277 (Figure 6). Distributions of mixing characteristics were slightly skewed toward the recurrent parent, OR943576. The average mix peak time of 3.79 minutes for progeny was significantly lower than N97S277 at 4.94 min, but not significantly different than OR943576 at 3.62 min. Short mix peak times were consistently near 2.5 min. No variation in mixing stability was identified within the population. Variation in mixing tolerance was normally distributed around the population average, which was slightly less than the midpoint of the parents. For each of the mixing parameters, distributions were slightly skewed toward the OR943576 parent. This was again likely due to the large number of backcross individuals within the population.

The variation among progeny for proportions of monomeric protein was normally distributed around the midpoint of the parents (Figure 7). Values ranged beyond that of the parental lines. Proportions of polymeric protein, albumins and globulins did not differ among progeny or parent cultivars.
Figure 8. Distribution of SDS sedimentation volumes for (A) Population 1, (B) Population 2, and (C) Population 3.

† Stephens
‡ Eltan
■ OR850513-8
☆ OR943576
● N97S277
♦ N96L1226
Population 1 Single Cross vs. Backcross

Backcrossing was used as a means to capture environmental adaptation and increase disease resistance in progeny. Significant differences (p ≤ 0.05) were observed in means of single cross and backcross progeny for PPO enzyme activity, kernel weight and diameter, flour yields, SDS sedimentation volumes, mix peak time, mixing stability, mixing tolerance, and protein composition (Figure 9). The differences were primarily related to the characteristics of the backcrossed parent, OR943576 as compared to N97S277.

The difference between single and backcross progeny PPO activity was statistically significant (Figure 9). However the mean change in PPO scores from 0.37 to 0.34 A₄₉₂ will have minimal impact on noodle color.

Backcross progeny had higher kernel weight, kernel diameter, and flour yields as compared to single cross progeny (Figure 9). An increase of 3.8 percentage points in flour yield in backcross progeny could be attributed to the recurrent parent, OR943576, which had higher relative break flour and total flour yields (9.2 and 5.1 %, respectively) as compared to N97S277.

Backcrossing decreased SDS sedimentation volumes in the progeny by 7.8 cc (11.2%). A 10.7% reduction in means for peak time between single cross and backcross progeny compares to a difference of 26.8 % between N97S277 and OR943576. Backcrossing to OR943576 reduced mixing tolerance. The mixing stability was increased in backcross progeny, but was not clearly attributed to the recurrent parent.
Figure 9. Means of grain yield, kernel weight, kernel diameter, kernel hardness, PPO activity, break flour yield, total flour yield, flour protein, SDS sedimentation volume, mix peak time, mixing stability, and mixing tolerance for single cross and backcross progeny in Populations 1 and 2. Bars for each trait with the same letter within the same population are not significantly different (p ≤ 0.05).
Relative proportions of polymeric protein and albumin and globulin were unchanged from the single cross to the backcross progeny (Table 5). However, the decrease in the relative proportion of monomeric protein for backcross progeny could be related to the recurrent parent, OR943576.

Backcrossing to OR943576, to capture agronomic traits, significantly reduced SDS sedimentation volume, mix peak time, and mixing tolerance. However, mean protein composition of the population was relatively unchanged.

**Population 2**

Parent cultivars for Population 2, N96L1226 and OR943576, differed significantly for all measured traits except kernel weight and hardness, flour yields, and mixing stability. Variation among progeny was observed in all parameters.

A high degree of variation in PPO enzyme activity was found among Population 2 progeny ranging from 0.18 to 1.20 A492 (Figure 4). Distribution of progeny was skewed toward the lower PPO recurrent parent, OR943576 (Figure 8). However, progeny were identified with enzyme activity beyond the range established by the parents. Flour protein and hardness values were normally distributed and exceeded the range of parents (Figure 5). Kernel weight and kernel diameter ranged from 41.22 to 54.19 mg and 2.74 to 3.37 mm, respectively. Significant variation was identified for total and break flour yield, ranging from 69.82 to 76.15% and 27.11 to 38.28%, respectively. Average flour yields were similar to parent midpoints.

There was a wide range in SDS sedimentation volumes among progeny. Values ranged from slightly lower than the low SDS parent OR943576, to higher
Table 5. Relative proportions of polymeric proteins, monomeric proteins, and albumins and globulins for single cross and backcross progeny as determined by HPLC for Populations 1 and 2.

<table>
<thead>
<tr>
<th>Comparison Class</th>
<th>n</th>
<th>Polymeric Protein</th>
<th>Monomeric Protein</th>
<th>Albumin and Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population Mean</td>
<td>158</td>
<td>41.55</td>
<td>40.13*</td>
<td>18.32</td>
</tr>
<tr>
<td>Single Cross Progeny Mean</td>
<td>44</td>
<td>41.24*</td>
<td>40.67*</td>
<td>18.09*</td>
</tr>
<tr>
<td>Backcross Progeny Mean</td>
<td>114</td>
<td>41.67*</td>
<td>39.92*</td>
<td>18.41*</td>
</tr>
<tr>
<td>Population Mean</td>
<td>135</td>
<td>40.20</td>
<td>40.72*</td>
<td>19.08</td>
</tr>
<tr>
<td>Single Cross Progeny Mean</td>
<td>39</td>
<td>39.84*</td>
<td>41.36*</td>
<td>18.81*</td>
</tr>
<tr>
<td>Backcross Progeny Mean</td>
<td>96</td>
<td>40.34*</td>
<td>40.47*</td>
<td>19.19*</td>
</tr>
</tbody>
</table>

* significant difference within comparison class at p ≤ 0.05
† means with the same letter in the same column in the same population are not significantly different at p ≤ 0.05
than N96L1226 (Figure 6). There also was significant variation among progeny for mixing characteristics. The range in mixing parameters among progeny exceeded the values established by the parents (Figure 6). On average, mix peak time, mixing stability, and mixing tolerance were reduced in the progeny as compared with the midpoint of the parents. This was in part due to the higher number of backcross progeny represented in the population.

Monomeric proteins, as a proportion of total protein, varied significantly among the progeny (Figure 7). Population means for polymeric, monomeric, and albumin and globulin proteins were similar to the midpoint of the parents, with the distribution skewed to higher monomeric protein levels.

**Population 2 single cross vs. backcross**

Single cross and backcross progeny differed in mean PPO activity, kernel weight and diameter, flour protein content and composition, SDS sedimentation volumes, mixing stability and tolerance (Figure 9). Differences generally reflect characteristics of the backcrossed OR943576 parent. There was no difference in mean grain yield, kernel hardness, flour yields, or mix peak time.

Backcross progeny showed a 0.10 A₄₉₂ (23%) increase in PPO activity. This was unexpected as the recurrent parent, OR943576, had lower average PPO activity than N96L1226. Kernel weights and diameters were lower in backcross progeny, but the difference did not affect flour yields. Backcross progeny had lower flour protein content than single cross progeny (8.33 as compared to 8.87 g kg⁻¹).
Backcrossing with the recurrent parent, OR943576, decreased SDS sedimentation volumes an average of 7.9 cc (10.5%) (Figure 8). Mix peak time for single cross and backcross progeny mix peak time means were not significantly different. Mixing stability was greater in backcross progeny while mixing tolerance was reduced.

Backcross progeny had lower levels of monomeric protein as compared to single cross progeny, similar to that of the recurrent parent, OR943576 (Figure 10). Relative proportions of polymeric protein, albumin and globulin were unchanged between single cross and backcross progeny.

Backcrossing for adaptation significantly reduced end-use quality of progeny in Population 2. Kernel characteristics, flour protein content, SDS sedimentation volume, and mixing tolerance were all reduced by backcrossing to OR943576. Quality of backcross progeny also was reduced by an unexpected increase in PPO activity.

**Population 3**

Parent cultivars, OR850513-8 and N97S277, differed for grain hardness, PPO, SDS sedimentation volume, and mixing characteristics. In this population, all progeny data were derived from a single cross. Significant variation was found among progeny for PPO activity, SKCS parameters, break flour yield, SDS sedimentation, mixing tolerance, and protein composition. There was no variation in total flour yield, flour protein content, mix peak time, or mixing stability.
Significant variation in PPO enzyme activity was observed among both parents and progeny of Population 3, even though progeny with high enzyme activity were not included in the study (Figure 4). Distribution of PPO activity for progeny was slightly skewed toward the low PPO parent, OR850513-8 (Figure 8). Grain hardness, kernel weight, and kernel diameter values were normally distributed with means similar or slightly higher than parent midpoints (Figure 4). Variation within the population was observed for break flour yield, but not total flour yield (Figure 5). The population mean for break flour was 1.16% higher than the parental midpoint. There was no significant variation in flour protein content among progeny (Figure 5).

The distribution of SDS sedimentation volumes for progeny in Population 3 was skewed to higher values (Figure 8). Most progeny had higher SDS sedimentation volumes than either parent and the progeny mean exceeded the midpoint of OR850513-8 and N97S277 (Figure 6).

Mix peak times and mixing stability did not vary among progeny in Population 3 (p ≤ 0.05) (Figure 6). However, progeny did vary significantly for mixing tolerance. Mixing tolerance was normally distributed around the midpoint of the parents with values for progeny exceeding the range established by the parents (Figure 6).

Significant variation was found among progeny for relative proportions of polymeric and monomeric protein (Figure 7). Compared with parental midpoints, there was a slight reduction in average polymeric protein and an increase in monomeric protein.
Summary over Populations

The parental combinations used to develop these populations provided sufficient genetic variability to develop acceptable quality HW cultivars for the PNW. Variations for PPO activity and kernel characteristics were observed in progeny of all three populations, with progeny means similar to that of the respective parental midpoints (Figure 4). Although flour protein content was lower than desired, a substantial number of progeny had quality attributes that met HW quality targets established by the Wheat Marketing Center in Portland, OR (U.S. Wheat Associates).

A wide range of variation in SDS sedimentation volumes, mix peak time, and mixing tolerance was found in all three populations (Figure 6). These measures of protein quality were highly correlated with flour protein content (Table 6). SDS sedimentation volumes and mixing tolerance were positively correlated with flour protein content, while mix peak time and mixing stability decreased as flour protein content increased. Both mix peak time and mixing tolerance had a positive correlation with SDS sedimentation volume.

As the relative proportion of monomeric proteins increased, there was an increase in SDS sedimentation volume and mixing tolerance and a decrease in mix peak time and stability. There was also a corresponding decrease in polymeric proteins. However, the proportion of monomeric proteins increased and the proportions of polymeric proteins, albumins and globulins decreased with increasing flour protein content, this makes it difficult to differentiate the relative role of flour protein content vs. protein composition.
Table 6. Correlation coefficients between flour yields, kernel characteristics, protein content and composition, PPO enzyme activity, SDS sedimentation volume, and mixing parameters.

<table>
<thead>
<tr>
<th></th>
<th>Flour Yield</th>
<th>Break Flour Yield</th>
<th>PPO</th>
<th>Kernel Hardness</th>
<th>Kernel Weight</th>
<th>Kernel Diameter</th>
<th>Protein Content</th>
<th>SDS</th>
<th>Mix Peak Time</th>
<th>Mixing Stability</th>
<th>Mixing Tolerance</th>
<th>Polymeric Protein</th>
<th>Monomeric Protein</th>
<th>Albumins and Globulins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour Yield</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Break Flour Yield</td>
<td>0.716</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>PPO</td>
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<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Kernel Hardness</td>
<td>0.032</td>
<td>-0.405</td>
<td>-0.039</td>
<td>1.000</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Kernel Weight</td>
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<td>-0.030</td>
<td>0.079</td>
<td>-0.253</td>
<td>1.000</td>
<td></td>
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<td>1.000</td>
</tr>
<tr>
<td>Kernel Diameter</td>
<td>0.184</td>
<td>-0.022</td>
<td>-0.017</td>
<td>-0.153</td>
<td>0.874</td>
<td>1.000</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Protein Content</td>
<td>0.131</td>
<td>-0.131</td>
<td>-0.035</td>
<td>0.297</td>
<td>0.015</td>
<td>0.171</td>
<td>1.000</td>
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<td>1.000</td>
</tr>
<tr>
<td>SDS</td>
<td>-0.091</td>
<td>-0.061</td>
<td>-0.042</td>
<td>0.087</td>
<td>-0.193</td>
<td>-0.004</td>
<td>0.478</td>
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<tr>
<td>Mix Peak Time</td>
<td>-0.126</td>
<td>0.074</td>
<td>0.023</td>
<td>-0.140</td>
<td>-0.211</td>
<td>-0.197</td>
<td>-0.260</td>
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<td>Mixing Stability</td>
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<td>0.108</td>
<td>-0.023</td>
<td>-0.037</td>
<td>-0.111</td>
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<td>0.039</td>
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<td>Mixing Tolerance</td>
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<td>-0.120</td>
<td>-0.045</td>
<td>0.071</td>
<td>-0.186</td>
<td>-0.046</td>
<td>0.145</td>
<td>0.668</td>
<td>0.480</td>
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<td>1.000</td>
<td></td>
<td></td>
<td>1.000</td>
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<td>Polymeric Protein</td>
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<td>-0.093</td>
<td>0.028</td>
<td>-0.021</td>
<td>-0.031</td>
<td>-0.147</td>
<td>-0.381</td>
<td>-0.244</td>
<td>0.073</td>
<td>0.032</td>
<td>-0.071</td>
<td>1.000</td>
<td></td>
<td>1.000</td>
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<tr>
<td>Monomeric Protein</td>
<td>-0.014</td>
<td>-0.101</td>
<td>-0.111</td>
<td>0.123</td>
<td>0.002</td>
<td>0.144</td>
<td>0.555</td>
<td>0.331</td>
<td>-0.102</td>
<td>-0.081</td>
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<td>-0.625</td>
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<td></td>
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<tr>
<td>Albumins and Globulins</td>
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<td>0.223</td>
<td>0.091</td>
<td>-0.112</td>
<td>0.035</td>
<td>0.013</td>
<td>-0.166</td>
<td>-0.080</td>
<td>0.027</td>
<td>0.053</td>
<td>-0.110</td>
<td>-0.490</td>
<td>-0.374</td>
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</tr>
</tbody>
</table>
Flour protein concentrations were relatively low in the study. Relationships observed among quality parameters in this study may differ if measured at higher average flour protein level.

There was a significant 'cost' of backcrossing with a lower-quality recurrent parent. Backcross progeny had lower average mix peak time and tolerance and lower SDS sedimentation volumes (Figure 9). Relative proportions of monomeric proteins also were decreased in backcross progeny, a function of the lower levels in the backcross parent, OR943576. The reduction in quality from backcrossing was sufficient that alternative crossing strategies should be considered, or at minimum, population size of backcross progeny should be increased. With regard to PPO activity, it was unclear why backcrossing with a lower PPO parent did not further reduce PPO activity of the progeny.

Determination of Protein Composition

The major HMW subunits of wheat (Payne and Lawrence, 1983) are represented in parents and segregating progeny (Table 7). Population 1 provides contrasts of HMW Glu-A1 HMW-GS: 2* and null, Glu-B1 HMW-GS: 6+8, 7+9, and 7, null and Glu-D1 HMW-GS: 2+12 and 5+10. Backcrossing increased the frequency of the Glu-A1 null as well as HMW-GS 6+8. Population 2 provides contrast of Glu-A1 HMW-GS: 1 and 2*, Glu-B1 HMW-GS: 6+8, 7+null and 7+9 and Glu-D1 HMW-GS: 2+12 and 5+10. Backcrossing in Population 2 increased the frequency of the 2* and 6+8 HMW-GS. Population 3 provides comparisons of Glu-A1 HMW-GS: 2* and null, Glu-B1 HMW-GS: 7+9 and 7+null and Glu-D1 HMW-
Table 7. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition between HMW-GS classes for pooled population data.

<table>
<thead>
<tr>
<th>Comparison Class</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Peak Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td>curve width at 6 min (% of total)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1 vs. 2*)</td>
<td>27</td>
<td>212</td>
<td>4.70*</td>
<td>-0.11</td>
<td>-0.01</td>
<td>-0.05</td>
</tr>
<tr>
<td>(1 vs. null)</td>
<td>27</td>
<td>105</td>
<td>12.40*</td>
<td>0.22</td>
<td>1.87*</td>
<td>-0.81*</td>
</tr>
<tr>
<td>(2* vs. null)</td>
<td>212</td>
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<td>7.70*</td>
<td>0.33*</td>
<td>1.88*</td>
<td>-0.77*</td>
</tr>
<tr>
<td>(1,2* vs. 2*)</td>
<td>19</td>
<td>212</td>
<td>7.40*</td>
<td>0.67*</td>
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<td>0.04</td>
</tr>
<tr>
<td>(1,2* vs. 1)</td>
<td>19</td>
<td>27</td>
<td>2.80</td>
<td>0.77*</td>
<td>2.43*</td>
<td>0.09</td>
</tr>
<tr>
<td>(1,2* vs. null)</td>
<td>19</td>
<td>105</td>
<td>15.10*</td>
<td>1.00*</td>
<td>4.41*</td>
<td>-0.72</td>
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<tr>
<td>(6+8 vs. 7+9)</td>
<td>180</td>
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<td>-13.60*</td>
<td>-0.47*</td>
<td>-4.03*</td>
<td>0.87*</td>
</tr>
<tr>
<td>(6+8 vs. 7+null)</td>
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<td>-2.64*</td>
<td>2.31*</td>
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<td>1.44*</td>
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<tr>
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<td>0.45</td>
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<td>(7+null vs. null)</td>
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<td>-0.31</td>
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<tr>
<td>(6+8, 7+null vs. 6+8)</td>
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<td>1.66*</td>
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<tr>
<td>(6+8, 7+null vs. 7+9)</td>
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<td>101</td>
<td>-7.90*</td>
<td>-0.41</td>
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<td>(6+8, 7+null vs. 7+null)</td>
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<td>34</td>
<td>-10.10*</td>
<td>0.35</td>
<td>-0.97</td>
<td>1.51*</td>
</tr>
<tr>
<td>(6+8, 7+null vs. null)</td>
<td>17</td>
<td>5</td>
<td>4.70</td>
<td>0.04</td>
<td>1.54</td>
<td>-0.89</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 6+8)</td>
<td>26</td>
<td>180</td>
<td>11.30*</td>
<td>0.56</td>
<td>2.34*</td>
<td>0.09</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 7+9)</td>
<td>26</td>
<td>101</td>
<td>-2.30*</td>
<td>0.08</td>
<td>-1.69*</td>
<td>0.97*</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 7+null)</td>
<td>26</td>
<td>34</td>
<td>-4.50</td>
<td>0.84</td>
<td>-0.29</td>
<td>2.41*</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. null)</td>
<td>26</td>
<td>5</td>
<td>10.3*</td>
<td>0.53</td>
<td>2.22</td>
<td>0.00</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 6+8, 7+null)</td>
<td>26</td>
<td>17</td>
<td>5.60*</td>
<td>0.49</td>
<td>0.68</td>
<td>0.89</td>
</tr>
<tr>
<td>(2+12 vs. 5+10)</td>
<td>237</td>
<td>126</td>
<td>-9.40*</td>
<td>-0.90*</td>
<td>-3.23*</td>
<td>0.65*</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
GS: 2+12 and 5+10. Not all HMW-GS were equally represented within the populations. Therefore, only banding patterns with at least 5 individuals exhibiting the pattern were used in statistical comparisons.

B-type LMW-GS were scored on a presence or absence basis for each of the populations, similar to Gupta and Shepard (1990). Five LMW-GS bands were polymorphic in Population 1 designated here as a, b, c, d, and e. Backcrossing increased the frequency of the b- and e-bands. Population 2 had four polymorphic bands: b, c, d, and e. Backcrossing did not affect the frequency of LMW-GS in Population 2. Five LMW-GS bands designated a, b, c, d, and e, were polymorphic in Population 3.

Population 1

Substitution of HMW-GS 5+10 over 2+10 were related to increased SDS sedimentation volumes by 9.9 cc, mix peak time by 69 sec and mixing tolerance by 4.39%. Similarly, substitution of 7+9 over 6+8 increased SDS sedimentation volumes by 11.8 cc, mix peak time by 40 sec and mixing tolerance by 3.34%. Relative proportions of protein fractions were not influenced by these HMW-GS (Table 8).

The presence of the 2* HMW-GS was related to higher SDS sedimentation volume and mix peak time as compared to the null of the locus. Mixing tolerance, however, was lower (Table 8). Presence of the null allele at the Glu-A1 locus was associated with decreased SDS sedimentation volumes (-5.6 cc) and mix peak time (-27 sec), but increased mixing tolerance (+2.38%). Relative proportions of
Table 8. Differences in SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition between allele 1 and allele 2 for progeny varying in HMW-GS in Population 1.

<table>
<thead>
<tr>
<th>Comparison Class</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Peak Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>allele 1</td>
<td>allele 2</td>
<td>cc</td>
<td>min</td>
<td>curve width at 6 min (% of total)</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>(allele 1 vs. allele 2)</td>
<td>81</td>
<td>77</td>
<td>5.60*</td>
<td>0.45*</td>
<td>-2.38*</td>
<td>-0.01</td>
</tr>
<tr>
<td>(2* vs. null)</td>
<td>102</td>
<td>32</td>
<td>-11.80*</td>
<td>-0.67*</td>
<td>-3.34*</td>
<td>0.10</td>
</tr>
<tr>
<td>(6+8 vs. 7+9)</td>
<td>5</td>
<td>102</td>
<td>-0.90</td>
<td>0.11</td>
<td>-0.11</td>
<td>1.08</td>
</tr>
<tr>
<td>(6+8, 7+null vs. 6+8)</td>
<td>5</td>
<td>32</td>
<td>-12.70*</td>
<td>-0.56</td>
<td>-3.44*</td>
<td>1.18</td>
</tr>
<tr>
<td>(6+8, 7+null vs. 7+9)</td>
<td>14</td>
<td>102</td>
<td>8.60*</td>
<td>0.67*</td>
<td>1.39</td>
<td>0.59</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 6+8)</td>
<td>14</td>
<td>32</td>
<td>-3.10</td>
<td>0.00</td>
<td>-1.95</td>
<td>0.69</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 7+9)</td>
<td>14</td>
<td>5</td>
<td>9.50*</td>
<td>0.56</td>
<td>1.49</td>
<td>-0.49</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 6+8, 7+null)</td>
<td>116</td>
<td>42</td>
<td>-9.90*</td>
<td>-1.15*</td>
<td>-4.39*</td>
<td>0.14</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
monomeric protein were 0.63% greater with the presence of the 2* HMW-GS than
the null at the locus. Increased monomeric protein proportions resulted in a decrease
in the relative proportion of albumin and globulin.

In general, the effects of 7+9 alone were approximately equal to 6+8 plus 7+9
in a heterozygous state (Table 8). These had higher average protein quality than
progeny with 6+8 or heterozygous progeny with 6+8 plus 7+null. Progeny with
HMW-GS 7+9 also had increased SDS sedimentation volume (+12.7 cc) and mixing
tolerance (from 9.9% to 13.4%) over progeny heterozygous for 6+8 plus 7+null. An
increase in the proportion of albumin and globulin was also attributed to the 7+9
HMW-GS.

Five LMW-bands were polymorphic in Population 1 (Table 9). The a-band
was present in 25% of the progeny, where as the b, c, and e-bands were present in
68, 78, and 63% of progeny, respectively. Only one individual did not possess the d-
band. These frequencies are a result of the greater number of backcross progeny
than single cross progeny in the population.

Influence of LMW-bands was determined by comparing means of progeny
with or without the given band (Table 9). Band-a contributed to significant increases
in SDS sedimentation volumes, from 62.7 to 71.9 cc, and mixing tolerance, from
10.24 to 12.65%, increased proportions of monomeric proteins, and reduced
albumins and globulins. Significant decreases in SDS sedimentation (67.1 to 63.4
c) and mixing tolerance (11.91 to 10.35%) were found associated with band-b.
Proportions of polymeric proteins also increased as monomeric proteins decreased.
Band-e was associated with decreased mixing tolerance (11.74 to 10.31%), mix peak
Table 9. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny varying for presence or absence of LMW-GS in Population 1.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Peak Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>a - - - -</td>
<td>40</td>
<td>118</td>
<td>7.40*</td>
<td>0.30</td>
<td>2.42*</td>
<td>-0.29</td>
</tr>
<tr>
<td>- b - - -</td>
<td>108</td>
<td>50</td>
<td>-3.80*</td>
<td>-0.24</td>
<td>-1.56*</td>
<td>0.50*</td>
</tr>
<tr>
<td>- - c - -</td>
<td>124</td>
<td>34</td>
<td>2.60</td>
<td>-0.11</td>
<td>0.13*</td>
<td>-0.04</td>
</tr>
<tr>
<td>- - d - e</td>
<td>157</td>
<td>1</td>
<td>-2.00</td>
<td>0.88</td>
<td>4.57</td>
<td>-3.62*</td>
</tr>
<tr>
<td>- - - - e</td>
<td>100</td>
<td>58</td>
<td>-2.70</td>
<td>-0.32*</td>
<td>-1.43*</td>
<td>0.61*</td>
</tr>
<tr>
<td>a b - - -</td>
<td>11</td>
<td>147</td>
<td>6.50*</td>
<td>0.26</td>
<td>1.88</td>
<td>0.20</td>
</tr>
<tr>
<td>a c - - -</td>
<td>30</td>
<td>128</td>
<td>9.40*</td>
<td>0.34</td>
<td>3.03*</td>
<td>-0.30</td>
</tr>
<tr>
<td>a - d - e</td>
<td>38</td>
<td>120</td>
<td>16.80*</td>
<td>0.28</td>
<td>2.49*</td>
<td>-0.38</td>
</tr>
<tr>
<td>a - - e</td>
<td>20</td>
<td>138</td>
<td>4.70*</td>
<td>-0.16</td>
<td>0.59</td>
<td>-0.11</td>
</tr>
<tr>
<td>- b c - -</td>
<td>95</td>
<td>63</td>
<td>-2.00</td>
<td>-0.13</td>
<td>-0.95</td>
<td>0.20</td>
</tr>
<tr>
<td>- b - d -</td>
<td>107</td>
<td>57</td>
<td>-3.80*</td>
<td>-0.21</td>
<td>-1.41*</td>
<td>0.39</td>
</tr>
<tr>
<td>- b - e</td>
<td>79</td>
<td>79</td>
<td>-2.50</td>
<td>-0.23</td>
<td>-1.18*</td>
<td>0.80*</td>
</tr>
<tr>
<td>- c d - e</td>
<td>124</td>
<td>34</td>
<td>2.60</td>
<td>-0.11</td>
<td>0.13*</td>
<td>0.06</td>
</tr>
<tr>
<td>- c - e</td>
<td>84</td>
<td>74</td>
<td>1.20</td>
<td>-0.30</td>
<td>-1.25*</td>
<td>0.45*</td>
</tr>
<tr>
<td>- - d e</td>
<td>99</td>
<td>59</td>
<td>-2.70</td>
<td>-0.29</td>
<td>-1.30*</td>
<td>0.51*</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
time (3.99 to 3.67 min), and increased proportion of polymeric protein. Effects of the \( d \)-band alone or in combination could not be adequately measured due to lack of polymorphism.

Variation associated with LMW-GS composition was more apparent when considered within each major HMW-GS background. With the 2+12 HMW-GS fixed (Table 10), SDS sedimentation volumes were increased in association with the \( a \)-band with \( a,c \)-band combination. Monomeric proteins increased an average of 1.4% and albumins and globulins decreased in association with band-\( a \) and all combinations of LMW-GS that include band-\( a \). A corresponding reduction in albumin and globulin was observed for each of these classes. Proportions of monomeric protein decreased an average of 0.53% in the presence of band-\( b \) as well as the \( b,c \)-band combination. The \( e \)-band was associated to a decrease in mixing tolerance from 10.41 to 9.27%. There was a similar decrease observed when \( e \) and \( b \)-bands and \( e \) and \( c \)-bands are paired. The relative proportion of polymeric protein was increased 41.20 to 41.80% with the presence of band-\( e \) and from 41.17 to 41.97% in association with the \( b,e \)-band combination.

When HMW-GS 5+10 was fixed (Table 11), variation in LMW proteins had relatively little influence on other quality traits. Mixing tolerance increased in association with the \( a \)-band, from 12.87% to 15.62%, as well as the \( a,c \)-band combination (13.12 to 15.56%). The \( a,e \) band combination was associated with a decrease in mix peak time by nearly a minute. Presence of band-\( a \) was associated with a decrease in the proportion of polymeric protein from 41.75 to 41.05%. The
Table 10. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 2+12 varying for presence or absence of LMW-GS in Population 1.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Peak Time</th>
<th>Mixing Tolerance</th>
<th>Polymeric Protein</th>
<th>Monomeric Protein</th>
<th>Albumin and Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td>curve width at 6 min (% of total)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>22</td>
<td>94</td>
<td>6.20*</td>
<td>0.07</td>
<td>0.55</td>
<td>-0.03</td>
<td>1.25*</td>
<td>-1.22*</td>
</tr>
<tr>
<td>- b</td>
<td>86</td>
<td>30</td>
<td>-3.60</td>
<td>-0.19</td>
<td>-0.80</td>
<td>0.36</td>
<td>-0.58*</td>
<td>0.22</td>
</tr>
<tr>
<td>- c</td>
<td>92</td>
<td>24</td>
<td>-6.40</td>
<td>-0.06</td>
<td>0.20</td>
<td>-0.07</td>
<td>-0.08</td>
<td>0.16</td>
</tr>
<tr>
<td>- d</td>
<td>115</td>
<td>1</td>
<td>-4.60</td>
<td>0.57</td>
<td>3.39</td>
<td>-3.59*</td>
<td>0.71</td>
<td>2.88</td>
</tr>
<tr>
<td>- e</td>
<td>76</td>
<td>40</td>
<td>-3.20</td>
<td>-0.28</td>
<td>-1.14*</td>
<td>0.59*</td>
<td>-0.22</td>
<td>0.37</td>
</tr>
<tr>
<td>a b</td>
<td>5</td>
<td>111</td>
<td>4.10</td>
<td>-0.10</td>
<td>0.25</td>
<td>1.03</td>
<td>1.65*</td>
<td>-2.68*</td>
</tr>
<tr>
<td>a c</td>
<td>14</td>
<td>102</td>
<td>9.30*</td>
<td>0.04</td>
<td>1.17</td>
<td>-0.07</td>
<td>1.77*</td>
<td>-0.70*</td>
</tr>
<tr>
<td>a d</td>
<td>21</td>
<td>95</td>
<td>6.10*</td>
<td>0.11</td>
<td>0.77</td>
<td>-0.23</td>
<td>1.34*</td>
<td>-1.10*</td>
</tr>
<tr>
<td>a e</td>
<td>12</td>
<td>104</td>
<td>3.60</td>
<td>0.03</td>
<td>-0.02</td>
<td>0.13</td>
<td>1.14*</td>
<td>-1.26*</td>
</tr>
<tr>
<td>b c</td>
<td>74</td>
<td>42</td>
<td>-1.80</td>
<td>-0.13</td>
<td>-0.42</td>
<td>0.09</td>
<td>-0.50*</td>
<td>0.42</td>
</tr>
<tr>
<td>b d</td>
<td>85</td>
<td>31</td>
<td>-3.40</td>
<td>-0.16</td>
<td>-0.63</td>
<td>0.19</td>
<td>-0.53*</td>
<td>0.34</td>
</tr>
<tr>
<td>b e</td>
<td>61</td>
<td>55</td>
<td>-2.70</td>
<td>-0.30</td>
<td>-1.01*</td>
<td>0.80*</td>
<td>-0.29</td>
<td>-0.50</td>
</tr>
<tr>
<td>c d</td>
<td>92</td>
<td>24</td>
<td>3.60</td>
<td>-0.06</td>
<td>0.20</td>
<td>-0.07</td>
<td>-0.08</td>
<td>0.16</td>
</tr>
<tr>
<td>c e</td>
<td>63</td>
<td>53</td>
<td>-1.40</td>
<td>-0.24</td>
<td>-1.06*</td>
<td>0.41</td>
<td>-0.24</td>
<td>-0.17</td>
</tr>
<tr>
<td>d e</td>
<td>75</td>
<td>41</td>
<td>-3.40</td>
<td>-0.25</td>
<td>-1.00*</td>
<td>0.45</td>
<td>-0.19</td>
<td>-0.26</td>
</tr>
</tbody>
</table>

* = significance at p \( \leq 0.05 \)
Table 11. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 5+10 varying for presence or absence of LMW-GS in Population 1.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Peak</th>
<th>Mixing</th>
<th>Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Time</td>
<td></td>
<td>curve width at 6</td>
<td>Polymeric</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>min (% of total)</td>
<td>Protein</td>
</tr>
<tr>
<td>a</td>
<td></td>
<td></td>
<td></td>
<td>-0.06</td>
<td>2.74*</td>
<td>-0.71*</td>
<td>0.49</td>
</tr>
<tr>
<td>- b</td>
<td></td>
<td></td>
<td></td>
<td>0.35</td>
<td>-0.47*</td>
<td>0.78*</td>
<td>-0.35</td>
</tr>
<tr>
<td>- c</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>0.62</td>
<td>0.38</td>
<td>-0.11</td>
</tr>
<tr>
<td>- d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- e</td>
<td></td>
<td></td>
<td></td>
<td>1.10</td>
<td>1.15</td>
<td>0.53</td>
<td>-0.20</td>
</tr>
<tr>
<td>a b</td>
<td></td>
<td></td>
<td></td>
<td>0.91</td>
<td></td>
<td></td>
<td>-0.48</td>
</tr>
<tr>
<td>a c</td>
<td></td>
<td></td>
<td></td>
<td>-0.13</td>
<td>2.44*</td>
<td>-0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>a d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Same as ‘a’</td>
</tr>
<tr>
<td>a e</td>
<td></td>
<td></td>
<td></td>
<td>2.60</td>
<td>-0.94*</td>
<td>0.43</td>
<td>-0.44</td>
</tr>
<tr>
<td>- b c</td>
<td></td>
<td></td>
<td></td>
<td>0.34</td>
<td>-0.50</td>
<td>0.45</td>
<td>-0.13</td>
</tr>
<tr>
<td>- b d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Same as ‘b’</td>
</tr>
<tr>
<td>- b e</td>
<td></td>
<td></td>
<td></td>
<td>0.90</td>
<td>0.31</td>
<td>-0.37</td>
<td>0.77*</td>
</tr>
<tr>
<td>- c d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Same as ‘c’</td>
</tr>
<tr>
<td>- c e</td>
<td></td>
<td></td>
<td></td>
<td>0.70</td>
<td>0.29</td>
<td>-1.14</td>
<td>0.53</td>
</tr>
<tr>
<td>- d e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Same as ‘e’</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
proportion of polymeric protein was increased in presence of band-b and b,e-band combination.

For progeny with HMW-GS 2* (Table 12), the LMW-GS a-band was again associated with increased SDS sedimentation volumes (+7.2 cc) and mixing tolerance (+2.39%). Combinations of the a-band with b or c-bands were also associated with increased SDS sedimentation volumes. Mixing tolerance increased more than 2.6% with the a,c-band combinations. Monomeric proteins were increased by at least 1.00% in relation to band-a and combinations with band-a. Presence of the b-band was associated to decreased SDS sedimentation volumes (on average -5.0 cc). Relative proportions of polymeric protein were increased by 0.75 to 0.99% in the presence of band-b and band-e, as well as the b,e-band combination. Reductions in proportions of albumin and globulin were complementary to increases in polymeric protein related to band-e and the combination of bands b and e.

When 2* was replaced by the null allele (Table 13), presence of the a and c-bands were related to increased SDS sedimentation volumes. An increase, from 39.73 to 40.22%, in the proportion of monomeric protein was related to the a-band. A similar increase was found with all LMW-GS band combinations that include the a-band. In contrast band-b and LMW-GS combinations of band-b reduced monomeric proteins from 40.15 to 39.64%. Band-b also was related to decreased mixing tolerance, from 10.60 to 9.18%. The exception for both was the a,b-band combination, the opposite effects of the a and b-bands appear to offset each other. Significant increases in the relative proportion of polymeric protein were found for band combinations a,b- and b,e-band combinations. The e-band was associated with
Table 12. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 2* varying for presence or absence of LMW-GS in Population 1.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS Time</th>
<th>Mixing Tolerance</th>
<th>Curve width at 6 min (% of total)</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td></td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>a</td>
<td>28</td>
<td>53</td>
<td>7.20*</td>
<td>0.09</td>
<td>2.39*</td>
<td>-0.51</td>
</tr>
<tr>
<td>b</td>
<td>55</td>
<td>26</td>
<td>-5.00*</td>
<td>-0.05</td>
<td>-1.58</td>
<td>0.76*</td>
</tr>
<tr>
<td>c</td>
<td>65</td>
<td>16</td>
<td>0.50</td>
<td>-0.16</td>
<td>-0.22</td>
<td>-0.21</td>
</tr>
<tr>
<td>d</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>49</td>
<td>32</td>
<td>-2.90</td>
<td>-0.34</td>
<td>-1.70</td>
<td>0.82*</td>
</tr>
<tr>
<td>a</td>
<td>10</td>
<td>71</td>
<td>4.30*</td>
<td>0.15</td>
<td>1.41</td>
<td>-0.18</td>
</tr>
<tr>
<td>c</td>
<td>21</td>
<td>57</td>
<td>7.80*</td>
<td>0.18</td>
<td>2.66*</td>
<td>-0.50</td>
</tr>
<tr>
<td>d</td>
<td>28</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td>Same as ‘a’</td>
</tr>
<tr>
<td>e</td>
<td>13</td>
<td>68</td>
<td>3.30</td>
<td>-0.49</td>
<td>-0.20</td>
<td>-0.28</td>
</tr>
<tr>
<td>b</td>
<td>49</td>
<td>32</td>
<td>-3.30</td>
<td>-0.01</td>
<td>-1.29</td>
<td>0.23</td>
</tr>
<tr>
<td>c</td>
<td>55</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td>Same as ‘b’</td>
</tr>
<tr>
<td>d</td>
<td>40</td>
<td>41</td>
<td>3.20</td>
<td>-0.13</td>
<td>-1.10</td>
<td>0.99*</td>
</tr>
<tr>
<td>e</td>
<td>65</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td>Same as ‘c’</td>
</tr>
<tr>
<td>d</td>
<td>41</td>
<td>40</td>
<td>-2.40</td>
<td>-0.47*</td>
<td>-1.68*</td>
<td>0.50</td>
</tr>
<tr>
<td>e</td>
<td>49</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td>Same as ‘d’</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
Table 13. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with a null at the \textit{Glu-A1} locus varying for presence or absence of LMW-GS in Population 1.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td>Curve width at 6 min (% of total)</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>a - - - -</td>
<td>12</td>
<td>65</td>
<td>4.60</td>
<td>0.39</td>
<td>0.88</td>
<td>0.06</td>
</tr>
<tr>
<td>b - - - -</td>
<td>53</td>
<td>24</td>
<td>-2.30</td>
<td>-0.42</td>
<td>-0.43*</td>
<td>0.22</td>
</tr>
<tr>
<td>c - - - -</td>
<td>59</td>
<td>18</td>
<td>3.90</td>
<td>-0.12</td>
<td>0.27</td>
<td>0.31</td>
</tr>
<tr>
<td>d - - - e</td>
<td>76</td>
<td>1</td>
<td>-4.90</td>
<td>0.65</td>
<td>3.35</td>
<td>-3.64*</td>
</tr>
<tr>
<td>e - - - -</td>
<td>51</td>
<td>26</td>
<td>-1.80</td>
<td>-0.23</td>
<td>-0.85</td>
<td>0.38</td>
</tr>
<tr>
<td>a b - - -</td>
<td>1</td>
<td>76</td>
<td>4.90</td>
<td>-0.65</td>
<td>-3.35</td>
<td>3.64*</td>
</tr>
<tr>
<td>a c - - -</td>
<td>6</td>
<td>71</td>
<td>9.10</td>
<td>0.26</td>
<td>1.51</td>
<td>0.22</td>
</tr>
<tr>
<td>a d - - -</td>
<td>11</td>
<td>66</td>
<td>4.50</td>
<td>0.48</td>
<td>1.34</td>
<td>-0.32</td>
</tr>
<tr>
<td>a e - - -</td>
<td>7</td>
<td>70</td>
<td>4.90</td>
<td>0.20</td>
<td>0.78</td>
<td>0.19</td>
</tr>
<tr>
<td>b c - - -</td>
<td>46</td>
<td>31</td>
<td>-0.70</td>
<td>-0.27</td>
<td>-0.59</td>
<td>0.18</td>
</tr>
<tr>
<td>b d - - -</td>
<td>52</td>
<td>25</td>
<td>-2.60</td>
<td>-0.38</td>
<td>-1.19</td>
<td>0.00</td>
</tr>
<tr>
<td>b e - - -</td>
<td>39</td>
<td>38</td>
<td>-1.70</td>
<td>-0.31</td>
<td>-1.18</td>
<td>0.50*</td>
</tr>
<tr>
<td>c d - - -</td>
<td>59</td>
<td>18</td>
<td>3.90</td>
<td>-0.12</td>
<td>0.27</td>
<td>0.31</td>
</tr>
<tr>
<td>c e - - -</td>
<td>43</td>
<td>34</td>
<td>0.80</td>
<td>-0.08</td>
<td>-0.49</td>
<td>0.40</td>
</tr>
<tr>
<td>d e - - -</td>
<td>50</td>
<td>27</td>
<td>2.00</td>
<td>-0.19</td>
<td>-0.64</td>
<td>0.17</td>
</tr>
</tbody>
</table>

* = significance at \(p \leq 0.05\)
a decreased proportion of monomeric proteins, from 40.11 to 39.65%, similar to the
decrease observed for the b,e and c,e-band combinations.

Effects of the a, b and e-bands were generally similar in the HMW-GS
backgrounds investigated. Increases in SDS sedimentation volume, mixing
tolerance, and proportion of monomeric protein were consistently associated with the
a-band. Decreased mixing tolerance and increased proportion of polymeric protein
were consistently associated with the b-band. The e-band was also consistently
related to an increased proportion of polymeric protein, however decreased mix peak
time and mixing tolerance did not vary within any of the HMW-GS backgrounds
investigated. The effect was only noticed in the population as a whole. Magnitude
of the effects varied in association with the dominating HMW-GS backgrounds. A
greater contribution of LMW-GS was observed in Glu-A1 as compared to Glu-D1
alleles, in 2* as compared to the null at the locus, and in 2+12 as compared to 5+10.

Population 2

In Population 2, the 5+10 HMW-GS was associated with increases in SDS
sedimentation volume (74.6 cc as compared to 67.6 cc), mix peak time (4.21 min as
compared to 3.15 min), and mixing tolerance (11.78% as compared to 9.37%)
comparison to progeny with HMW-GS 2+12. There was, however, no difference in
protein composition among progeny that vary in Glu-D1 alleles (Table 14). HMW-
GS 1 increased SDS sedimentation volumes by almost 10 cc and mixing tolerance by
2.4% over progeny with HMW-GS 2* or the Glu-A1 null. Progeny that were
Table 14. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition between allele 1 and allele 2 for progeny varying in HMW-GS in Population 2

<table>
<thead>
<tr>
<th>Comparison Class</th>
<th>N1</th>
<th>N2</th>
<th>SDS Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>curve width at 6 min (% of total)</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>(allele 1 vs. allele 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1 vs. 2*)</td>
<td>27</td>
<td>68</td>
<td>9.90*</td>
<td>0.14</td>
<td>2.42*</td>
</tr>
<tr>
<td>(1 vs. null)</td>
<td>27</td>
<td>21</td>
<td>9.20*</td>
<td>0.71*</td>
<td>2.48*</td>
</tr>
<tr>
<td>(2* vs. null)</td>
<td>68</td>
<td>21</td>
<td>-0.80</td>
<td>0.56*</td>
<td>0.06</td>
</tr>
<tr>
<td>(1,2* vs. 2*)</td>
<td>19</td>
<td>68</td>
<td>12.70*</td>
<td>0.91*</td>
<td>4.85*</td>
</tr>
<tr>
<td>(1,2* vs. 1)</td>
<td>19</td>
<td>27</td>
<td>2.80</td>
<td>0.77*</td>
<td>2.43*</td>
</tr>
<tr>
<td>(1,2* vs. null)</td>
<td>19</td>
<td>21</td>
<td>11.90*</td>
<td>1.48*</td>
<td>4.91*</td>
</tr>
<tr>
<td>(6+8 vs. 7+9)</td>
<td>78</td>
<td>31</td>
<td>-8.30</td>
<td>-0.27</td>
<td>-3.62*</td>
</tr>
<tr>
<td>(6+8, 7+null vs. 6+8)</td>
<td>12</td>
<td>78</td>
<td>6.60*</td>
<td>0.08</td>
<td>2.82*</td>
</tr>
<tr>
<td>(6+8, 7+null vs. 7+9)</td>
<td>12</td>
<td>31</td>
<td>-1.80</td>
<td>-0.19</td>
<td>-0.79</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 6+8)</td>
<td>13</td>
<td>78</td>
<td>13.30*</td>
<td>0.26</td>
<td>3.37*</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 7+9)</td>
<td>13</td>
<td>31</td>
<td>4.90</td>
<td>-0.01</td>
<td>-0.25</td>
</tr>
<tr>
<td>(6+8, 7+9 vs. 6+8, 7+null)</td>
<td>13</td>
<td>12</td>
<td>6.70</td>
<td>0.18</td>
<td>0.54</td>
</tr>
<tr>
<td>(2+12 vs. 5+10)</td>
<td>83</td>
<td>52</td>
<td>-7.90*</td>
<td>-1.13*</td>
<td>-2.60*</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
heterozygous for 1, 2* had increased SDS sedimentation volumes and mixing
tolerance as compared with those with a single allele or null (Table 14). The relative
proportion of polymeric protein was increased in association with the heterozygous
combination of 1 and 2* vs. 1 alone, while the proportion of monomeric protein
decreased (Table 14).

The presence of HMW-GS 7+9 was associated with increased mixing
tolerance as compared with progeny with HMW-GS 6+8. Heterozygous
combinations of 6+8 with 7+9 or 6+8 with 7+null increased in SDS sedimentation
volumes and mixing tolerance compared to those with 6+8 alone (Table 14).

There was a little significant variation in LMW-GS composition in
Population 2 (Table 15). Four LMW bands were polymorphic in progeny. Band-b
was present in 87% of the progeny, band-c was present in 78% of progeny, band-d
was present in 96% of the progeny, and band-e was present in 95% of the progeny.
There was no clear effect of LMW bands on SDS sedimentation volumes, mixing
parameters or the proportions of polymeric or monomeric proteins. Relative
proportions of albumin and globulin decreased in the presence of the b,d and c,d-
band combinations.

Progeny with the 2+12 HMW-GS (Table 16) showed an increase in the
relative proportion of polymeric protein with the presence of b-band or b,e-band
combination. When 5+10 was fixed, there was no significant variation associated
with LMW banding patterns (Table 17). The LMW-GS b,c-band combinations
reduced the relative proportion of monomeric protein in progeny with HMW-GS 1
Table 15. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny varying for presence or absence of LMW-GS in Population 2.

<table>
<thead>
<tr>
<th>LMW-GS band(s) present</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Peak Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.55</td>
</tr>
<tr>
<td>- b</td>
<td>118</td>
<td>17</td>
<td>-0.60</td>
<td>-0.12</td>
<td>-0.73</td>
<td></td>
</tr>
<tr>
<td>- c</td>
<td>105</td>
<td>3</td>
<td>1.10</td>
<td>-0.18</td>
<td>0.17</td>
<td>0.49</td>
</tr>
<tr>
<td>- d</td>
<td>130</td>
<td>5</td>
<td>1.30</td>
<td>0.10</td>
<td>1.65</td>
<td>-0.29</td>
</tr>
<tr>
<td>- e</td>
<td>128</td>
<td>7</td>
<td>5.10</td>
<td>-0.13</td>
<td>1.63</td>
<td>0.67</td>
</tr>
<tr>
<td>- b c</td>
<td>97</td>
<td>38</td>
<td>-1.50</td>
<td>-0.22</td>
<td>-0.77</td>
<td>0.45</td>
</tr>
<tr>
<td>- b d</td>
<td>113</td>
<td>22</td>
<td>-0.10</td>
<td>-0.07</td>
<td>-0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>- b e</td>
<td>116</td>
<td>19</td>
<td>0.10</td>
<td>-0.14</td>
<td>0.08</td>
<td>0.75</td>
</tr>
<tr>
<td>- c d</td>
<td>100</td>
<td>35</td>
<td>1.20</td>
<td>-0.14</td>
<td>0.46</td>
<td>0.39</td>
</tr>
<tr>
<td>- c e</td>
<td>99</td>
<td>36</td>
<td>1.00</td>
<td>-0.26</td>
<td>0.29</td>
<td>0.52</td>
</tr>
<tr>
<td>- d e</td>
<td>123</td>
<td>12</td>
<td>3.70</td>
<td>-0.02</td>
<td>1.71</td>
<td>0.28</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
Table 16. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 2+12 varying for presence or absence of LMW-GS in Population 2.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS cc</th>
<th>Mix Peak Time Min</th>
<th>Mixing Tolerance Curve width at 6 min (% of total)</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Protein</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Protein</td>
<td>% of total</td>
</tr>
<tr>
<td>- b - - - -</td>
<td>73</td>
<td>10</td>
<td>1.90</td>
<td>0.09</td>
<td>0.20</td>
<td>1.20*</td>
</tr>
<tr>
<td>- - c - -</td>
<td>66</td>
<td>17</td>
<td>4.90</td>
<td>-0.04</td>
<td>0.87</td>
<td>0.41</td>
</tr>
<tr>
<td>- - - - d -</td>
<td>80</td>
<td>3</td>
<td>-0.60</td>
<td>0.09</td>
<td>2.37</td>
<td>-1.15</td>
</tr>
<tr>
<td>- - - - e</td>
<td>78</td>
<td>5</td>
<td>6.40</td>
<td>0.12</td>
<td>0.93</td>
<td>0.97</td>
</tr>
<tr>
<td>- b c - -</td>
<td>62</td>
<td>21</td>
<td>1.40</td>
<td>-0.10</td>
<td>0.04</td>
<td>0.71</td>
</tr>
<tr>
<td>- b - d -</td>
<td>70</td>
<td>13</td>
<td>1.30</td>
<td>-0.04</td>
<td>0.79</td>
<td>0.66</td>
</tr>
<tr>
<td>- b - - e</td>
<td>71</td>
<td>12</td>
<td>1.90</td>
<td>-0.09</td>
<td>0.47</td>
<td>1.23*</td>
</tr>
<tr>
<td>- - c d -</td>
<td>63</td>
<td>20</td>
<td>4.10</td>
<td>-0.02</td>
<td>1.24</td>
<td>0.15</td>
</tr>
<tr>
<td>- - c - e</td>
<td>63</td>
<td>20</td>
<td>4.30</td>
<td>-0.05</td>
<td>0.67</td>
<td>0.59</td>
</tr>
<tr>
<td>- - - d e</td>
<td>75</td>
<td>8</td>
<td>3.90</td>
<td>0.11</td>
<td>1.56</td>
<td>0.17</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
Table 17. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 5+10 varying for presence or absence of LMW-GS in Population 2.

<table>
<thead>
<tr>
<th>LWM-GS band present</th>
<th>N1</th>
<th>N2</th>
<th>SDS Time cc</th>
<th>Mix Peak Time min</th>
<th>Mixing Tolerance curve width at 6 min (% of total)</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>- b - - -</td>
<td>45</td>
<td>7</td>
<td>-3.60</td>
<td>0.01</td>
<td>-1.86</td>
<td>-0.40</td>
</tr>
<tr>
<td>- - c - -</td>
<td>39</td>
<td>13</td>
<td>-2.90</td>
<td>0.19</td>
<td>-0.37</td>
<td>0.59</td>
</tr>
<tr>
<td>- - d -</td>
<td>50</td>
<td>2</td>
<td>4.40</td>
<td>0.16</td>
<td>0.68</td>
<td>0.98</td>
</tr>
<tr>
<td>- - e</td>
<td>50</td>
<td>2</td>
<td>-0.80</td>
<td>-0.80</td>
<td>2.45</td>
<td>-0.03</td>
</tr>
<tr>
<td>- b c - -</td>
<td>35</td>
<td>17</td>
<td>-3.90</td>
<td>-0.11</td>
<td>-1.22</td>
<td>0.08</td>
</tr>
<tr>
<td>- b d -</td>
<td>43</td>
<td>9</td>
<td>-1.80</td>
<td>0.05</td>
<td>-1.34</td>
<td>-0.07</td>
</tr>
<tr>
<td>- b e</td>
<td>45</td>
<td>7</td>
<td>3.20</td>
<td>-0.19</td>
<td>-0.68</td>
<td>-0.07</td>
</tr>
<tr>
<td>- c d -</td>
<td>37</td>
<td>15</td>
<td>-1.90</td>
<td>-0.14</td>
<td>-0.22</td>
<td>0.72</td>
</tr>
<tr>
<td>- c e</td>
<td>36</td>
<td>16</td>
<td>-2.10</td>
<td>-0.28</td>
<td>0.31</td>
<td>0.42</td>
</tr>
<tr>
<td>- d e</td>
<td>48</td>
<td>4</td>
<td>1.80</td>
<td>-0.33</td>
<td>1.63</td>
<td>0.50</td>
</tr>
</tbody>
</table>
(Table 18). With HMW-GS 2* there were reductions of albumin and globulin associated with the b-band and b,d and b,e-band combinations (Table 19).

Contributions of the LMW-GS were minor in Population 2. The overall lack of variation in LMW-GS composition may have contributed to the lack of variation in SDS sedimentation volume, mixing parameters, and protein molecular weight distribution in the population.

**Population 3**

Presence of the 5+10 allele compared to the 2+12 allele increased SDS sedimentation volume from 79.4 to 82.8 cc and mixing tolerance from 12.66 to 14.35% over progeny with the 2+12 allele combination (Table 20). The 5+10 allele also increased the proportion of monomeric protein from 41.73 to 43.26% and decreased polymeric protein from 39.46 to 38.22%, relative to 2+12. The Glu-AI null was associated with increased SDS sedimentation volumes (+8.2 cc) over the 2* mean (Table 20). The null also increased the relative proportion of albumin and globulin. Substitution of 7+9 with 7+null decreased SDS sedimentation volumes by 3.4 cc, mix peak time by 0.89 min and mixing tolerance by 2.66%. The 7, null locus increased the proportion of monomeric protein (43.02%) and decreased albumin and globulin (18.32%), as compared to 7+9 (41.90% and 19.00%, respectively) (Table 20). All five LMW-bands were polymorphic in Population 3 (Table 21). Band-e had the largest influence on mixing parameters. Mix peak time was decreased in the presence of band-e and the b,e-band combination. When e-band was combined with the a, b or c-band, mixing tolerance decreased. The a,e and c,e-band combinations
Table 18. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 1 varying for presence or absence of LMW-GS in Population 2.

<table>
<thead>
<tr>
<th>LWM-GS band present</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Peak Time</th>
<th>Mixing Tolerance curve width at 6 min (% of total)</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>24</td>
<td>3</td>
<td>-0.30</td>
<td>0.38</td>
<td>-1.42</td>
<td>1.35</td>
</tr>
<tr>
<td>-</td>
<td>21</td>
<td>6</td>
<td>-0.80</td>
<td>-0.11</td>
<td>0.86</td>
<td>0.64</td>
</tr>
<tr>
<td>-</td>
<td>27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>24</td>
<td>3</td>
<td>4.90</td>
<td>-0.17</td>
<td>0.75</td>
<td>0.79</td>
</tr>
<tr>
<td>-</td>
<td>18</td>
<td>9</td>
<td>-0.80</td>
<td>0.03</td>
<td>0.03</td>
<td>1.11</td>
</tr>
<tr>
<td>-</td>
<td>24</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>22</td>
<td>5</td>
<td>1.70</td>
<td>0.11</td>
<td>0.16</td>
<td>1.19</td>
</tr>
<tr>
<td>-</td>
<td>21</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>18</td>
<td>9</td>
<td>1.50</td>
<td>-0.15</td>
<td>1.00</td>
<td>0.85</td>
</tr>
<tr>
<td>-</td>
<td>24</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
Table 19. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 2* varying for presence or absence of LMW-GS in Population 2.

<table>
<thead>
<tr>
<th>LWM-GS band present</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Peak Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>LWM-GS band present</td>
<td>N1</td>
<td>N2</td>
<td>SDS</td>
<td>Mix Peak Time</td>
<td>Mixing Tolerance</td>
<td>curve width at 6 min (% of total)</td>
</tr>
<tr>
<td>- b - -</td>
<td>59</td>
<td>9</td>
<td>0.40</td>
<td>-0.14</td>
<td>-0.70</td>
<td>0.46</td>
</tr>
<tr>
<td>- - c -</td>
<td>52</td>
<td>16</td>
<td>0.70</td>
<td>0.09</td>
<td>-0.53</td>
<td>0.34</td>
</tr>
<tr>
<td>- - - d</td>
<td>64</td>
<td>4</td>
<td>-3.70</td>
<td>-0.20</td>
<td>-0.35</td>
<td>-0.13</td>
</tr>
<tr>
<td>- - - e</td>
<td>65</td>
<td>3</td>
<td>5.00</td>
<td>-0.72</td>
<td>0.99</td>
<td>0.23</td>
</tr>
<tr>
<td>- b c -</td>
<td>50</td>
<td>18</td>
<td>9.00</td>
<td>-0.23</td>
<td>-1.09</td>
<td>0.16</td>
</tr>
<tr>
<td>- b - d</td>
<td>55</td>
<td>13</td>
<td>-1.00</td>
<td>-0.18</td>
<td>-0.65</td>
<td>0.30</td>
</tr>
<tr>
<td>- b - e</td>
<td>60</td>
<td>8</td>
<td>1.30</td>
<td>-0.39</td>
<td>-0.07</td>
<td>0.72</td>
</tr>
<tr>
<td>- c d -</td>
<td>48</td>
<td>20</td>
<td>0.30</td>
<td>-0.13</td>
<td>-0.56</td>
<td>0.26</td>
</tr>
<tr>
<td>- c - e</td>
<td>50</td>
<td>18</td>
<td>0.60</td>
<td>-0.39</td>
<td>-0.75</td>
<td>0.19</td>
</tr>
<tr>
<td>- - d e</td>
<td>61</td>
<td>7</td>
<td>0.10</td>
<td>-0.44</td>
<td>0.24</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* = significance at $p \leq 0.05$
Table 20. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition between allele 1 and allele 2 for progeny varying in HMW-GS in Population 3.

<table>
<thead>
<tr>
<th>Comparison Class</th>
<th>N1</th>
<th>N2</th>
<th>SDS Mix Peak Time</th>
<th>Mixing Tolerance Curve width at 6 min (% of total)</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2* vs. null)</td>
<td>63</td>
<td>7</td>
<td>-8.20*</td>
<td>0.39</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.06*</td>
</tr>
<tr>
<td>(7+9 vs. 7+null)</td>
<td>33</td>
<td>37</td>
<td>3.40*</td>
<td>0.89*</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.13*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.68*</td>
</tr>
<tr>
<td>(2+12 vs. 5+10)</td>
<td>38</td>
<td>32</td>
<td>-3.40*</td>
<td>-0.29</td>
<td>1.24*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.53*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.28</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
Table 21. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny varying for presence or absence of LMW-GS in Population 3.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS Mix Time</th>
<th>Mixing Tolerance Curve width at 6 min (% of total)</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td>Polymeric Protein Monomeric Protein Albumin and Globulin</td>
</tr>
<tr>
<td>a</td>
<td>47</td>
<td>23</td>
<td>-2.10</td>
<td>0.21</td>
<td>-0.68</td>
</tr>
<tr>
<td>- b</td>
<td>53</td>
<td>17</td>
<td>2.10</td>
<td>-0.21</td>
<td>-0.95</td>
</tr>
<tr>
<td>- - c</td>
<td>55</td>
<td>15</td>
<td>-1.10</td>
<td>0.20</td>
<td>0.59</td>
</tr>
<tr>
<td>- - - D</td>
<td>58</td>
<td>12</td>
<td>1.00</td>
<td>1.24</td>
<td>0.69</td>
</tr>
<tr>
<td>- - - - E</td>
<td>34</td>
<td>36</td>
<td>-1.80</td>
<td>-0.48*</td>
<td>-1.73*</td>
</tr>
<tr>
<td>a b</td>
<td>32</td>
<td>38</td>
<td>-0.20</td>
<td>0.08</td>
<td>-0.99</td>
</tr>
<tr>
<td>a c</td>
<td>43</td>
<td>27</td>
<td>-1.90</td>
<td>0.06</td>
<td>-0.39</td>
</tr>
<tr>
<td>a - - D</td>
<td>35</td>
<td>35</td>
<td>-1.30</td>
<td>0.47*</td>
<td>0.10</td>
</tr>
<tr>
<td>a - - - E</td>
<td>20</td>
<td>50</td>
<td>-420*</td>
<td>-0.27</td>
<td>-1.87*</td>
</tr>
<tr>
<td>- b c</td>
<td>42</td>
<td>28</td>
<td>0.90</td>
<td>0.07</td>
<td>-0.07</td>
</tr>
<tr>
<td>- b - D</td>
<td>41</td>
<td>29</td>
<td>2.20</td>
<td>0.13</td>
<td>0.01</td>
</tr>
<tr>
<td>- b - - E</td>
<td>28</td>
<td>42</td>
<td>0.70</td>
<td>-0.54*</td>
<td>-1.17</td>
</tr>
<tr>
<td>- - c D</td>
<td>43</td>
<td>27</td>
<td>-0.20</td>
<td>0.44</td>
<td>1.17</td>
</tr>
<tr>
<td>- - c - E</td>
<td>20</td>
<td>50</td>
<td>-3.30*</td>
<td>-0.47</td>
<td>-1.50*</td>
</tr>
<tr>
<td>- - - D E</td>
<td>23</td>
<td>47</td>
<td>-1.10</td>
<td>-0.17</td>
<td>-1.06</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
also decreased SDS sedimentation volumes. The combination of \( a,d \)-bands had a positive association with mix peak time.

Variation of protein composition was primarily affected by LMW-GS bands-\( a, c, \) and \( d \), and combinations of these bands (Table 21). Polymeric protein decreased in association with \( a \) or the \( a,c \)-band combination. Band-\( c \) alone, and in combination with the \( a \) or \( e \)-bands, was associated with an increased proportion of monomeric protein. Band-\( d \) either alone or in combination with the \( b \) or \( e \)-bands, was associated with decreased monomeric proteins. The relative proportion of albumin and globulin was increased by the \( d \)-band, \( a,d \) and \( c,d \) combinations. The \( b,e \)-band combination also was related to decreased albumin and globulin.

When progeny with the HMW-GS 2+12 were evaluated (Table 22), bands-\( a \) and \( e \) were associated with reductions in mixing tolerance of 16.19 to 13.74% and 15.12 to 13.23%, respectively. Similar reductions were found with combinations of \( a,e \) and \( c,e \). Combinations of the \( a,e \) and \( c,e \)-bands also decreased SDS sedimentation volumes by more than 6 cc. The \( b \)-band and \( b,d \)-band combination were associated with an increase of approximately 5cc in SDS sedimentation volume. However, among 2+12 progeny, there was no significant change in mix peak time or protein composition associated with variation in LMW composition (Table 22).

In the presence of HMW 5+10 (Table 23), LMW-GS composition was associated with changes in protein molecular weight distributions as well as mixing characteristics. Band-\( d \) and the \( c,d \)-band combination were associated with a 0.80 to 1.00 min increase in mix peak time and increased mixing tolerance. These also were
Table 22. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 2+12 varying for presence or absence of LMW-GS in Population 3.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS (cc)</th>
<th>Mix Time (min)</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Curves width at 6 min (% of total)</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>a - - - - -</td>
<td>22</td>
<td>16</td>
<td>-3.50</td>
<td>0.42</td>
<td>-1.71*</td>
<td>-1.14</td>
</tr>
<tr>
<td>a b - - -</td>
<td>29</td>
<td>9</td>
<td>5.10*</td>
<td>0.05</td>
<td>0.41</td>
<td>0.66</td>
</tr>
<tr>
<td>- - c - -</td>
<td>27</td>
<td>11</td>
<td>-2.30</td>
<td>0.19</td>
<td>0.17</td>
<td>-0.56</td>
</tr>
<tr>
<td>- - - d -</td>
<td>34</td>
<td>4</td>
<td>1.70</td>
<td>-0.06</td>
<td>1.07</td>
<td>0.08</td>
</tr>
<tr>
<td>- - - e</td>
<td>19</td>
<td>19</td>
<td>-2.50</td>
<td>-0.31</td>
<td>-1.97*</td>
<td>0.31</td>
</tr>
<tr>
<td>a b - - -</td>
<td>14</td>
<td>24</td>
<td>0.20</td>
<td>0.40</td>
<td>-1.18</td>
<td>-0.75</td>
</tr>
<tr>
<td>a - c - -</td>
<td>19</td>
<td>19</td>
<td>-3.50</td>
<td>0.16</td>
<td>-1.27</td>
<td>-1.22</td>
</tr>
<tr>
<td>a - d -</td>
<td>18</td>
<td>20</td>
<td>-2.80</td>
<td>0.38</td>
<td>-1.27</td>
<td>-1.09</td>
</tr>
<tr>
<td>a - e</td>
<td>10</td>
<td>28</td>
<td>-6.40*</td>
<td>0.19</td>
<td>-2.45*</td>
<td>-0.04</td>
</tr>
<tr>
<td>- b c -</td>
<td>20</td>
<td>18</td>
<td>1.50</td>
<td>0.17</td>
<td>0.43</td>
<td>0.01</td>
</tr>
<tr>
<td>- b d -</td>
<td>25</td>
<td>13</td>
<td>4.80*</td>
<td>0.01</td>
<td>0.78</td>
<td>0.57</td>
</tr>
<tr>
<td>- b - e</td>
<td>15</td>
<td>23</td>
<td>2.10</td>
<td>-0.25</td>
<td>-0.60</td>
<td>0.38</td>
</tr>
<tr>
<td>- c d</td>
<td>23</td>
<td>15</td>
<td>-1.30</td>
<td>0.14</td>
<td>0.57</td>
<td>-0.45</td>
</tr>
<tr>
<td>- c E</td>
<td>9</td>
<td>29</td>
<td>-6.30*</td>
<td>-0.31</td>
<td>-2.14*</td>
<td>-0.30</td>
</tr>
<tr>
<td>- - d E</td>
<td>15</td>
<td>23</td>
<td>2.00</td>
<td>-0.35</td>
<td>-1.64</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
Table 23. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 5+10 varying for presence or absence of LMW-GS in Population 3.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS Mix Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td>Curve width at 6 min (% of total)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>a</td>
<td>25</td>
<td>7</td>
<td>-2.20</td>
<td>-0.30</td>
<td>-0.12</td>
</tr>
<tr>
<td>- b</td>
<td>24</td>
<td>8</td>
<td>-1.10</td>
<td>-0.50</td>
<td>-2.45*</td>
</tr>
<tr>
<td>- c - d</td>
<td>28</td>
<td>4</td>
<td>-1.40</td>
<td>0.01</td>
<td>0.23</td>
</tr>
<tr>
<td>- e</td>
<td>24</td>
<td>8</td>
<td>2.10</td>
<td>1.00*</td>
<td>2.13*</td>
</tr>
<tr>
<td>a b - c - d e</td>
<td>15</td>
<td>17</td>
<td>-0.60</td>
<td>-0.66*</td>
<td>-1.34</td>
</tr>
<tr>
<td>a - c - d e</td>
<td>18</td>
<td>14</td>
<td>-2.00</td>
<td>-0.40</td>
<td>-1.59</td>
</tr>
<tr>
<td>a - d - e</td>
<td>24</td>
<td>8</td>
<td>-2.20</td>
<td>-0.29</td>
<td>-0.28</td>
</tr>
<tr>
<td>a - d - e</td>
<td>17</td>
<td>15</td>
<td>0.10</td>
<td>0.55</td>
<td>1.52</td>
</tr>
<tr>
<td>a - d - e</td>
<td>10</td>
<td>22</td>
<td>-2.30</td>
<td>-0.81*</td>
<td>-1.47</td>
</tr>
<tr>
<td>- b c - d e</td>
<td>22</td>
<td>10</td>
<td>-1.10</td>
<td>-0.20</td>
<td>-1.45</td>
</tr>
<tr>
<td>- b - d e</td>
<td>16</td>
<td>16</td>
<td>0.70</td>
<td>0.38</td>
<td>-0.24</td>
</tr>
<tr>
<td>- b - e</td>
<td>13</td>
<td>19</td>
<td>-1.10</td>
<td>-0.88*</td>
<td>-1.88*</td>
</tr>
<tr>
<td>- c d - e</td>
<td>20</td>
<td>12</td>
<td>1.00</td>
<td>0.80*</td>
<td>1.81*</td>
</tr>
<tr>
<td>- c - e</td>
<td>11</td>
<td>21</td>
<td>-1.40</td>
<td>-0.72*</td>
<td>-1.36</td>
</tr>
<tr>
<td>- d e</td>
<td>8</td>
<td>24</td>
<td>1.70</td>
<td>0.21</td>
<td>0.45</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
associated with a decrease in monomeric proteins and an increase in albumin and globulins. Band-\textit{b} and \textit{b,e} combinations were associated with decreased mixing tolerance. Mix peak time was decreased by more than 0.6 min with band-\textit{e}, or combinations of \textit{b,e} or \textit{c,e}. The combinations also were associated with an increase in monomeric proteins and a decrease in albumin and globulins. The \textit{a,e}-band combination was associated with an increase in monomeric proteins and decrease in albumins and globulins, which contributed to an decrease in mix peak time of 0.81 min.

There were insufficient progeny with the \textit{Glu-IA} null allele to examine contributions of LMW bands within this HMW background. For progeny with the \textit{2*} allele, band-\textit{a} and combination \textit{a,c} were associated with a reduction in the relative proportion of polymeric proteins (Table 24). An increase in mix peak time of approximately 0.5 min and increased proportions of albumin and globulins was associated with the \textit{a,d} and \textit{c,d}-band combinations. Band-\textit{b} was associated with decreased mixing tolerance and reduced proportions of albumins and globulins. The combination of bands \textit{b} and \textit{d} was associated with lower levels of monomeric proteins. Band-\textit{e} and the combination \textit{b,e} reduced mix peak time by more than 0.5 min. Mix, tolerance and the proportion of albumin and globulin also decreased. The \textit{c,e}-band combination was associated with changes in protein compositions, increasing monomeric proteins and decreasing albumins and globulins. Mix peak time was reduced by 0.53 min in association with the \textit{c,e}-band combination, but the difference was not statistically significant.
Table 24. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 2* varying for presence or absence of LMW-GS in Population 3.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Curve width at 6 min (% of total)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>a - - - - -</td>
<td>44</td>
<td>19</td>
<td>-0.50</td>
<td>0.29</td>
<td>-0.17</td>
<td>-1.08*</td>
</tr>
<tr>
<td>- b - - - -</td>
<td>48</td>
<td>15</td>
<td>-0.30</td>
<td>-0.25</td>
<td>-1.76*</td>
<td>0.48</td>
</tr>
<tr>
<td>- - c - -</td>
<td>50</td>
<td>13</td>
<td>0.10</td>
<td>0.25</td>
<td>0.99</td>
<td>-0.85</td>
</tr>
<tr>
<td>- - - d -</td>
<td>51</td>
<td>12</td>
<td>2.10</td>
<td>0.57</td>
<td>1.46</td>
<td>0.71</td>
</tr>
<tr>
<td>- - - - e</td>
<td>30</td>
<td>33</td>
<td>-1.10</td>
<td>-0.55*</td>
<td>-1.65*</td>
<td>0.15</td>
</tr>
<tr>
<td>a b - - - -</td>
<td>31</td>
<td>32</td>
<td>-0.60</td>
<td>0.11</td>
<td>-1.08</td>
<td>-0.59</td>
</tr>
<tr>
<td>a - c - -</td>
<td>40</td>
<td>23</td>
<td>-0.10</td>
<td>0.12</td>
<td>0.17</td>
<td>-1.18*</td>
</tr>
<tr>
<td>a - - d -</td>
<td>32</td>
<td>31</td>
<td>0.90</td>
<td>0.59*</td>
<td>0.76</td>
<td>-0.47</td>
</tr>
<tr>
<td>a - - e</td>
<td>18</td>
<td>45</td>
<td>-2.50</td>
<td>-0.30</td>
<td>-1.50*</td>
<td>-0.12</td>
</tr>
<tr>
<td>- b c - -</td>
<td>39</td>
<td>24</td>
<td>0.20</td>
<td>0.07</td>
<td>-0.44</td>
<td>-0.16</td>
</tr>
<tr>
<td>- b - d -</td>
<td>36</td>
<td>27</td>
<td>1.10</td>
<td>0.17</td>
<td>-0.38</td>
<td>0.80</td>
</tr>
<tr>
<td>- b - - e</td>
<td>26</td>
<td>37</td>
<td>-0.90</td>
<td>-0.64*</td>
<td>-1.63*</td>
<td>0.07</td>
</tr>
<tr>
<td>- - c d -</td>
<td>38</td>
<td>25</td>
<td>1.40</td>
<td>0.53*</td>
<td>1.62*</td>
<td>-0.12</td>
</tr>
<tr>
<td>- - c e</td>
<td>18</td>
<td>45</td>
<td>-1.50</td>
<td>-0.53</td>
<td>-1.09</td>
<td>-0.50</td>
</tr>
<tr>
<td>- - - d e</td>
<td>19</td>
<td>44</td>
<td>0.50</td>
<td>-0.19</td>
<td>-0.78</td>
<td>0.52</td>
</tr>
</tbody>
</table>

* = significance at $p \leq 0.05$
Progeny with HMW-GS 7+9 (Table 25) had lower SDS sedimentation volume when the \(a,e\)-band combination was present. A decrease in mix peak time of nearly 2.4 min was associated with the \(d\)-band in a 7+9 background. However, there were no significant differences in mixing tolerance or protein composition associated with LMW-GS composition among progeny carrying the HMW-GS 7+9.

LMW composition had a relatively greater influence on quality for progeny with HMW-GS 7+null (Table 26). Lower proportions of polymeric proteins and increased monomeric proteins were associated with band \(a\), band \(c\), and the combinations \(a,b\) and \(a,c\). Although mix peak time was increased slightly, there was no significant changes in mixing properties or SDS sedimentation volume. The combination of bands \(a,d\) was associated with an increase of 0.75 min in mix peak time and increased proportions of albumin and globulins. The \(b,c\)-band combination was associated with increased mixing tolerance and increased proportions of monomeric proteins. Band-\(d\) was associated with a decrease in monomeric proteins and increase in albumin and globulins. When band-\(d\) was combined with either \(b\) or \(e\), the decrease in monomeric proteins was associated with an increase in polymeric proteins. Band-\(e\) and \(b,e\) were associated with a decrease of 0.9 min in mix peak time. Proportions of polymeric proteins were increased at the expense of albumins and globulins.

Interactions between HMW and LMW-GS were more evident in Population 3. Associations of LMW-GS with variation in mixing properties and protein compositions were more numerous and of greater magnitude when examined within each HMW-GS background. LMW-GS composition had relatively greater influence
Table 25. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 7+9 varying for presence or absence of LMW-GS in Population 3.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS Mix Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cc  min</td>
<td>curve width at 6 min (% of total)</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>a - - - -</td>
<td>24</td>
<td>9</td>
<td>-0.60 0.18</td>
<td>-1.00</td>
<td>-0.64</td>
</tr>
<tr>
<td>- b - - -</td>
<td>28</td>
<td>5</td>
<td>0.20 0.16</td>
<td>-1.24</td>
<td>0.69</td>
</tr>
<tr>
<td>- c - - -</td>
<td>27</td>
<td>6</td>
<td>0.20 0.01</td>
<td>0.84</td>
<td>-0.39</td>
</tr>
<tr>
<td>- - d - e</td>
<td>22</td>
<td>11</td>
<td>5.70 -2.38*</td>
<td>0.81</td>
<td>-0.68</td>
</tr>
<tr>
<td>- - e</td>
<td>20</td>
<td>13</td>
<td>-0.20 0.27</td>
<td>-1.02</td>
<td>0.03</td>
</tr>
<tr>
<td>a b - - -</td>
<td>19</td>
<td>14</td>
<td>-0.60 0.34</td>
<td>-1.86</td>
<td>-0.10</td>
</tr>
<tr>
<td>a - c - -</td>
<td>24</td>
<td>9</td>
<td>0.20 0.11</td>
<td>0.04</td>
<td>-0.83</td>
</tr>
<tr>
<td>a - d - e</td>
<td>13</td>
<td>20</td>
<td>0.00 -0.08</td>
<td>-0.89</td>
<td>-0.47</td>
</tr>
<tr>
<td>a - e</td>
<td>12</td>
<td>21</td>
<td>-3.10* 0.29</td>
<td>-1.96</td>
<td>0.35</td>
</tr>
<tr>
<td>- b c - -</td>
<td>22</td>
<td>11</td>
<td>-0.10 0.13</td>
<td>-0.57</td>
<td>0.32</td>
</tr>
<tr>
<td>- b d - e</td>
<td>17</td>
<td>16</td>
<td>0.90 -0.12</td>
<td>-1.10</td>
<td>0.58</td>
</tr>
<tr>
<td>- b - e</td>
<td>18</td>
<td>15</td>
<td>0.50 0.36</td>
<td>-0.47</td>
<td>-0.03</td>
</tr>
<tr>
<td>- c d - e</td>
<td>16</td>
<td>17</td>
<td>1.00 -0.30</td>
<td>0.90</td>
<td>-0.46</td>
</tr>
<tr>
<td>- c e</td>
<td>14</td>
<td>19</td>
<td>-0.90 0.25</td>
<td>-0.50</td>
<td>-0.54</td>
</tr>
<tr>
<td>- d e</td>
<td>10</td>
<td>23</td>
<td>0.40 0.00</td>
<td>-0.96</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
Table 26. Differences in mean SDS sedimentation volume, mix peak time, mixing tolerance, and protein composition for progeny with HMW-GS 7+null varying for presence or absence of LMW-GS in Population 3.

<table>
<thead>
<tr>
<th>LMW-GS bands present</th>
<th>N1</th>
<th>N2</th>
<th>SDS</th>
<th>Mix Time</th>
<th>Mixing Tolerance</th>
<th>Relative Proportion of Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cc</td>
<td>min</td>
<td>curve width at 6 min (% of total)</td>
<td>Polymeric Protein</td>
</tr>
<tr>
<td>a - - - -</td>
<td>23</td>
<td>14</td>
<td>-3.1</td>
<td>0.51</td>
<td>0.46</td>
<td>-1.31*</td>
</tr>
<tr>
<td>- b - - -</td>
<td>25</td>
<td>12</td>
<td>8.4*</td>
<td>-0.32</td>
<td>1.38</td>
<td>0.18</td>
</tr>
<tr>
<td>- - c - -</td>
<td>28</td>
<td>9</td>
<td>-2.3</td>
<td>0.65</td>
<td>0.83</td>
<td>-1.41*</td>
</tr>
<tr>
<td>- - d - -</td>
<td>36</td>
<td>1</td>
<td>-1.9</td>
<td>0.35</td>
<td>0.35</td>
<td>0.70</td>
</tr>
<tr>
<td>- - - - e</td>
<td>14</td>
<td>23</td>
<td>-1.9</td>
<td>-0.91*</td>
<td>-1.38*</td>
<td>0.73*</td>
</tr>
<tr>
<td>a b - - -</td>
<td>13</td>
<td>24</td>
<td>1.9</td>
<td>0.25</td>
<td>1.10</td>
<td>-0.97*</td>
</tr>
<tr>
<td>a - c - -</td>
<td>19</td>
<td>18</td>
<td>-3.1</td>
<td>0.51</td>
<td>0.46</td>
<td>-1.31*</td>
</tr>
<tr>
<td>a - d - -</td>
<td>22</td>
<td>15</td>
<td>-4.3</td>
<td>0.75*</td>
<td>0.06</td>
<td>-0.44</td>
</tr>
<tr>
<td>a - e - -</td>
<td>8</td>
<td>29</td>
<td>-4.2</td>
<td>-0.45</td>
<td>-0.99</td>
<td>-0.27</td>
</tr>
<tr>
<td>- b c - -</td>
<td>20</td>
<td>17</td>
<td>3.3</td>
<td>0.25</td>
<td>1.35*</td>
<td>-0.84*</td>
</tr>
<tr>
<td>- b d - -</td>
<td>24</td>
<td>13</td>
<td>2.7</td>
<td>0.15</td>
<td>0.39</td>
<td>0.71*</td>
</tr>
<tr>
<td>- b e - -</td>
<td>10</td>
<td>27</td>
<td>2.9</td>
<td>-0.91*</td>
<td>-0.43</td>
<td>0.60</td>
</tr>
<tr>
<td>- c d - -</td>
<td>27</td>
<td>10</td>
<td>-3.0</td>
<td>0.70</td>
<td>0.18</td>
<td>-0.22</td>
</tr>
<tr>
<td>- c e - -</td>
<td>6</td>
<td>31</td>
<td>-3.3</td>
<td>-0.49</td>
<td>-0.84</td>
<td>-0.15</td>
</tr>
<tr>
<td>- d e - -</td>
<td>13</td>
<td>24</td>
<td>-3.2</td>
<td>-0.49</td>
<td>-1.52*</td>
<td>1.24*</td>
</tr>
</tbody>
</table>

* = significance at p ≤ 0.05
on mixing properties and protein composition in a 5+10 background as compared with 2+12. For HMW alleles at the Glu-BI locus, LMW-GS contributions also were of greater magnitude with 7+null as compared with a 7+9 background. Associations of individual LMW bands with protein and mixing quality were generally consistent in direction, but varied in magnitude. No dominant LMW-GS was identified.

**Summary of Variation in Protein Composition over Populations**

Influence of Glu-DI and Glu-BI alleles on protein quality and mixing properties were generally consistent over the three populations. Progeny with the Glu-DI allele, HMW-GS 5+10, as compared to 2+12, consistently had higher SDS sedimentation volume, mix peak time, mixing tolerance, and proportions of polymeric proteins, as well as decreased proportions of monomeric proteins. This is consistent with past research showing that HMW-GS 5+10 is associated with greater dough strength than 2+12 for hard wheat bread quality (Payne et al., 1984, Singh et al., 1990a, Luo et al., 2001).

The influence of Glu-BI alleles on protein quality was relatively consistent over populations with 7+9 > 7+null > 6+8. This agrees with previous research by Payne et al. (1984). HMW-GS 7+9 was associated with increased SDS sedimentation volume and mixing tolerance as compared with HMW-GS 7+null and increased mix peak time as compared with HMW-GS 6+8. Heterogenous progeny (7+9 and 6+8 or 7+null and 6+8) were found in Populations 1 and 2. Mixing characteristics of these progeny were generally intermediate of the respective
progeny that were homogeneous for HMW-GS. Protein molecular weight distribution was relatively unchanged by Glu-B1 alleles in Populations 1 and 2. In Population 3, HMW-GS 7+9 was associated with increased proportions of monomeric proteins and decreased proportions of albumins and globulins. Relative to progeny with HMW-GS 7+null, however, there was no effect on quality parameters.

Glu-A1 allele composition varied among the populations. For Population 1, progeny with HMW-GS 2* were associated with increased SDS sedimentation volumes, mix peak time, and mixing tolerance as compared to those with the null allele, Glu-A1c. This was similar to that found by Sontag-Strohm et al. (1996). For Population 2, progeny that were heterogeneous at the Glu-A1 locus (HMW-GS 1,2*) had longer mix peak time and greater mixing tolerance as compared to those homogeneous for HMW-GS 1. HMW-GS 1 was associated with greater SDS sedimentation volume and mixing tolerance as compared with HMW-GS 2*. Progeny with HMW-GS 1 or 2*, had improved protein quality as compared to progeny with the Glu-A1c null allele.

Contribution of LMW-GS varied over populations. Increased quality was associated with presence of higher molecular weight bands. Band-a, which had the lowest mobility, was associated with increased SDS sedimentation volume and mixing tolerance. Band-a also was associated with an increase in the proportion of monomeric protein and decrease in polymeric proteins. Band-b, which had intermediate band mobility, was associated with reduced mixing tolerance and increased proportions of polymeric protein. This corresponded to a decrease in
monomeric protein and albumin and globulin. The c and d-bands were nearly fixed in the populations and there was little variation in quality associated with these two bands. Band-e was associated with reduced mix peak time and mixing tolerance. An increase in polymeric protein, with corresponding reductions in proportions of monomeric proteins and albumin and globulins, also were related to band-e. There was little evidence of interactions among LMW-GS band pairs. When present, band-a was generally dominant to other bands.

The contributions of LMW-GS varied over populations depending on the presence (or absence) of major HMW-GS. Influences of LMW-GS on mixing and protein quality were most frequent and of greatest magnitude in HMW-GS backgrounds associated with lower quality, such as Glu-D1 HMW-GS 2+12, Glu-Al HMW-GS 2*, or Glu-B1 HMW-GS 7+null. By combining HMW-GS 2+12 with LMW-GS band-a in Population 1, SDS sedimentation volume and mixing tolerance were nearly equal to that of progeny with HMW-GS 5+10. The exception was in Population 3, where influences of LMW-GS were of relatively greater magnitude in a 5+10 as compared with a 2+12 HMW-GS background.

Interactions of LMW-GS with Glu-A1 alleles were not as easy to assess due to unequal class numbers. In Population 1, changes associated with bands-a, b, and e were similar in HMW-GS 1, 2, or null backgrounds.

The interactions of LMW-GS with Glu-B1 HMW-GS 7+9 and 7+null were investigated only in Population 3. In the presence of HMW-GS 7+9, band-d was associated with reduced mix peak time. No other associations with LMW-GS were found in this background. LMW-GS composition has relatively greater influence on
quality in the HMW-GS 7+null background. Band-\(a\) and band-\(c\) were related to decreased proportions of polymeric proteins and increased monomeric proteins. Band-\(b\) was associated with reduced SDS sedimentation volumes and lower levels of albumin and globulin. Band-\(e\) was associated with decreased mix peak time, mixing tolerance, and reduced proportion of albumins and globulins.

Influences of LMW-GS on protein and mixing quality were inconsistent among populations. This was due, in part, to the larger influence of HMW-GS (Payne et al., 1987; Singh et al., 1990a; Sontag-Strohm et al. 1996) which may have masked LMW-GS contributions or interactions between the alleles. Linkage of Glu-3 and Gli-1 alleles (Jackson et al., 1996) may also be playing a role in the inconsistencies. Furthermore, SE-HPLC does not allow precise differentiation between polymeric and monomeric proteins, particularly LMW-GS as compared to HMW-gliadins (Larroque, 1997).

LMW-GS effects were influenced by HMW-GS composition. However, these interactions were associated with changes in magnitude of effects rather than changes in rank. The presence of band-\(a\), either alone or in paired combinations, was associated with improved protein quality and mixing properties as compared to either band-\(b\) or \(e\). The improvement in quality associated to LMW-GS band-\(a\) was not sufficient enough to fully compensate for the differences in quality associated with Glu-\(D1\) alleles. Nonetheless, some progeny with HMW-GS 2+12 and band-\(a\) had protein and mixing quality exceeding progeny with HMW-GS 5+10, indicating that interactions with LMW-GS were compensating for the normal reduction in dough quality associated with HMW-GS 2+12.
Conclusions

Crosses between hard wheat germplasm from the Great Plains possessing strong gluten, good baking quality, and superior protein quality with PNW germplasm having good noodle color and color stability provided sufficient genetic variability to develop HW cultivars with acceptable end-use quality. There was however, a significant ‘cost’ of backcrossing to the OR943576 parent. Reductions in SDS sedimentation volumes, mix peak time, and mixing tolerance were all associated with backcrossing as related to the lower protein quality of OR943576. Decreased proportions of monomeric proteins also were observed in backcross progeny. These results suggest that, when backcrossing with a parent that has deficiencies in protein quality, a larger population size may be necessary to identify progeny with acceptable end-use quality. It also may also be valuable to consider other crossing strategies to increase the frequency of superior quality progeny.

HMW-GS have been proven useful indicators of protein quality. The superior bread-making properties of hexaploid wheat are largely attributed to alleles at the Glu-ID locus. At this locus, HMW-GS 5+10 is considered to have higher bread-making quality as compared to HMW-GS 2+12. The superiority of 5+10 was confirmed in this study in progeny comparisons from all three populations. However, several progeny in this study with HMW-GS 2+12 exceeded the quality parameter means of progeny with HMW-GS 5+10, indicating potential interactions with, or positive contributions of LMW-GS.
Variation in LMW-GS composition was associated to changes in SDS sedimentation volumes, mix peak time, mixing tolerance, and protein molecular weight distribution. Yet, the influence of individual LMW-GS on mixing and protein quality varied over the populations. Contributions of LMW-GS also varied depending on background HMW-GS composition. However, these changes were generally in magnitude of effects rather than rank. Effects of LMW-GS were more apparent when examined within lower quality HMW-GS backgrounds such as 2+12 or 7+null. In general, bands-a and -e were associated with larger changes in protein quality and composition, as compared to bands b, c, or d. When combinations of LMW-GS were examined, influences on quality were either intermediate, or similar to that of the dominant LMW-GS allele, such as band-a or -e.

Improvements in protein quality will lead to improved end-product quality of HW varieties grown in the PNW. Manipulation of HMW-GS composition has been a successful strategy for breeders to improve protein quality of hard wheats used for bread making. In this study, LMW-GS composition and interactions between LMW-GS and HMW-GS had significant influences on dough mixing properties and protein composition. The results suggest that further improvements in protein quality can be achieved by understanding and manipulating variation in LMW-GS composition.


Regnier, S., R. Holcomb, and P. Rayas-Duarte. Relating wheat quality to end-product quality. Oklahoma State University Food Technology Fact Sheet FAPC-129. (Available at www.fapc.okstate.edu)


Appendix
Appendix A. Mean values and ranges of grain yield, kernel weight, kernel diameter, kernel hardness, and PPO enzyme activity for Population 1, 2 and 3.

<table>
<thead>
<tr>
<th>Comparison Class</th>
<th>n</th>
<th>Yield (kg Ha(^{-1}))</th>
<th>Kernel Weight (mg)</th>
<th>Kernel Diameter (mm)</th>
<th>Kernel Hardness (HI)</th>
<th>PPO (A_{492})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population Mean</td>
<td>158</td>
<td>7239.28*</td>
<td>41.68*</td>
<td>2.76*</td>
<td>70.20*</td>
<td>0.35*</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>5070.60</td>
<td>31.61</td>
<td>2.34</td>
<td>58.25</td>
<td>0.19</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>9788.93</td>
<td>49.99</td>
<td>3.09</td>
<td>89.08</td>
<td>0.54</td>
</tr>
<tr>
<td>Single Cross Progeny Mean</td>
<td>44</td>
<td>7296.73(^a)</td>
<td>40.65(^b)</td>
<td>2.69(^b)</td>
<td>70.41(^*)</td>
<td>0.37(^*)</td>
</tr>
<tr>
<td>Backcross Progeny Mean</td>
<td>114</td>
<td>7217.11(^a)</td>
<td>42.08(^*)</td>
<td>2.78(^*)</td>
<td>70.12(^*)</td>
<td>0.34(^*)</td>
</tr>
</tbody>
</table>

* Population Mean             | 135 | 6897.63*                | 42.67*             | 2.82*                | 71.50*               | 0.42*           |
| Minimum                     |     | 5056.37                 | 41.22              | 2.74                 | 74.39                | 0.32            |
| Maximum                     |     | 8902.91                 | 54.19              | 3.37                 | 87.98                | 0.56            |
| Single Cross Progeny Mean   | 39  | 6814.80\(^a\)          | 44.17\(^*\)        | 2.94\(^*\)           | 71.67\(^*\)          | 0.35\(^*\)      |
| Backcross Progeny Mean      | 96  | 6931.30\(^a\)          | 42.06\(^*\)        | 2.77\(^*\)           | 71.10\(^*\)          | 0.45\(^*\)      |

* Population Mean             | 70  | 6845.95                 | 40.27*             | 2.78*                | 71.16*               | 0.35*           |
| Minimum                     |     | 5077.72                 | 35.32              | 2.48                 | 58.53                | 0.21            |
| Maximum                     |     | 8351.37                 | 49.50              | 3.23                 | 86.09                | 0.54            |

* Significant difference within comparison class at p \(\leq 0.05\)

\(^{\dagger}\) Means with the same letter in the same column in the same population are not significantly different at p \(\leq 0.05\)
Appendix B. Mean values and ranges of break flour yield, total flour yield, grain protein content, SDS sedimentation volume, mix peak time, mixing stability and mixing tolerance for Populations 1, 2, and 3.

<table>
<thead>
<tr>
<th>Comparison Class</th>
<th>n</th>
<th>Break Flour Yield</th>
<th>Total Flour Yield</th>
<th>Protein</th>
<th>SDS Time</th>
<th>Mixing Stability</th>
<th>Mixing Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>g kg⁻¹</td>
<td>cc</td>
<td>min</td>
<td>slope of curve</td>
<td>curve width at 6 min (of total)</td>
</tr>
<tr>
<td>Population Mean</td>
<td>158</td>
<td>30.43*</td>
<td>68.04*</td>
<td>7.96</td>
<td>64.6*</td>
<td>3.79*</td>
<td>5.62*</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>19.61</td>
<td>50.90</td>
<td>6.83</td>
<td>45.1</td>
<td>0.78</td>
<td>-24.48</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>39.54</td>
<td>75.46</td>
<td>9.44</td>
<td>85.5</td>
<td>6.80</td>
<td>-1.89</td>
</tr>
<tr>
<td>Single Cross Progeny Mean</td>
<td>44</td>
<td>29.61*</td>
<td>66.23b</td>
<td>7.96a</td>
<td>70.2*</td>
<td>4.11a</td>
<td>13.15*</td>
</tr>
<tr>
<td>Backcross Progeny Mean</td>
<td>114</td>
<td>30.74**</td>
<td>68.74*</td>
<td>7.95a</td>
<td>62.4*</td>
<td>3.67b</td>
<td>-5.49*</td>
</tr>
<tr>
<td>Population Mean</td>
<td>135</td>
<td>32.17*</td>
<td>71.61*</td>
<td>8.48</td>
<td>69.8*</td>
<td>3.56*</td>
<td>10.24*</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>27.11</td>
<td>69.82</td>
<td>8.01</td>
<td>63.0</td>
<td>2.75</td>
<td>-7.13</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>38.28</td>
<td>76.15</td>
<td>10.52</td>
<td>86.0</td>
<td>7.29</td>
<td>-2.08</td>
</tr>
<tr>
<td>Single Cross Progeny Mean</td>
<td>39</td>
<td>32.37**</td>
<td>71.91*</td>
<td>8.87a</td>
<td>75.4**</td>
<td>3.44**</td>
<td>11.41*</td>
</tr>
<tr>
<td>Backcross Progeny Mean</td>
<td>96</td>
<td>32.09**</td>
<td>71.51**</td>
<td>8.33b</td>
<td>67.5**</td>
<td>3.61**</td>
<td>9.77b*</td>
</tr>
<tr>
<td>Population Mean</td>
<td>70</td>
<td>31.74*</td>
<td>69.56</td>
<td>8.92</td>
<td>81.0*</td>
<td>3.64</td>
<td>5.27</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>27.49</td>
<td>65.91</td>
<td>7.17</td>
<td>58.0</td>
<td>1.68</td>
<td>-11.71</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>36.19</td>
<td>73.96</td>
<td>10.47</td>
<td>87.5</td>
<td>6.37</td>
<td>-2.02</td>
</tr>
</tbody>
</table>

* significant difference within comparison class at \( p \leq 0.05 \)

† means with the same letter in the same column in the same population are not significantly different at \( p \leq 0.05 \)
Appendix C. Mean values and ranges of relative proportions of polymeric protein, monomeric protein, albumin and globulin as determined by SE-HPLC for Populations 1, 2 and 3.

<table>
<thead>
<tr>
<th>Comparison Class</th>
<th>n</th>
<th>Polymeric Protein</th>
<th>Monomeric Protein</th>
<th>Albumin and Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population 1 Mean</td>
<td>158</td>
<td>41.55</td>
<td>40.13*</td>
<td>18.32</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>35.66</td>
<td>35.70</td>
<td>13.77</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>45.15</td>
<td>45.51</td>
<td>28.64</td>
</tr>
<tr>
<td>Single Cross Progeny</td>
<td>44</td>
<td>41.24*</td>
<td>40.67**</td>
<td>18.09*</td>
</tr>
<tr>
<td>Backcross Progeny Mean</td>
<td>114</td>
<td>41.67*</td>
<td>39.92**</td>
<td>18.41*</td>
</tr>
<tr>
<td>Population 2 Mean</td>
<td>135</td>
<td>40.20</td>
<td>40.72*</td>
<td>19.08</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>35.85</td>
<td>37.17</td>
<td>16.54</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>43.98</td>
<td>47.60</td>
<td>23.35</td>
</tr>
<tr>
<td>Single Cross Progeny</td>
<td>39</td>
<td>39.84*</td>
<td>41.36**</td>
<td>18.81*</td>
</tr>
<tr>
<td>Backcross Progeny Mean</td>
<td>96</td>
<td>40.34*</td>
<td>40.47**</td>
<td>19.19*</td>
</tr>
<tr>
<td>Population 3 Mean</td>
<td>70</td>
<td>38.89*</td>
<td>42.43*</td>
<td>18.68</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>35.62</td>
<td>35.37</td>
<td>15.65</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>48.98</td>
<td>46.48</td>
<td>21.24</td>
</tr>
</tbody>
</table>

* significant difference within comparison class at p ≤ 0.05
† means with the same letter in the same column in the same population are not significantly different at p ≤ 0.05
Appendix D. SDS-PAGE banding patterns of parent and check cultivars.

<table>
<thead>
<tr>
<th>Parent</th>
<th>HMW Allele Composition</th>
<th>LMW Allele Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR943576</td>
<td>2*, 6+8, 2+12</td>
<td>b, d, e</td>
</tr>
<tr>
<td>OR850513-8</td>
<td>2*, 7+null, 2+12</td>
<td>a, b, c, e</td>
</tr>
<tr>
<td>N97S277</td>
<td>2*, 7+9, 5+10</td>
<td>a, b, c, d</td>
</tr>
<tr>
<td>N96L1226</td>
<td>1, 7+9, 5+10</td>
<td>b, c, d, e</td>
</tr>
<tr>
<td>Stephens</td>
<td>7+null, 2+12</td>
<td>a, c, e</td>
</tr>
<tr>
<td>Eltan</td>
<td>1, 7+9, 5+10</td>
<td>a, b, c, e</td>
</tr>
</tbody>
</table>