

**A STUDY OF THE RHEOLOGICAL PROPERTIES AND GLUTEN
PROTEIN COMPONENTS ASSOCIATED WITH ENHANCED
BAKING QUALITY IN DURUM WHEAT**
(Triticum turgidum L. var. durum)

A Thesis Submitted to the College of
Graduate Studies and Research
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the Department of Plant Sciences
University of Saskatchewan
Saskatoon, Saskatchewan, Canada

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Abstract

Durum wheat (*Triticum turgidum* L. var. *durum*, $2n = 4x = 28$, AABB genomes) is used predominantly for semolina and pasta products, but there is increasing interest in using durum for bread-making to provide alternative markets during periods of overproduction. The goal of this study was to characterize the bread-making quality of durum wheat cultivars and emmer (*Triticum turgidum* L. var. *dicoccum*, $2n = 4x = 28$) derived breeding lines derived from crosses of durum wheat with an Emmer land race ‘97Emmer19’ from Iran. Emmer-derived breeding lines were evaluated along with three high quality bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD genomes) cultivars and seven durum wheat cultivars across three environments in replicated yield trials in the 2005 and 2006 growing seasons. Four 1AS.1AL-1DL translocation lines which carry the *Glu-D1d* allele [high molecular weight glutenin subunit (HMW-GS) pair *1Dx5+1Dy10*] from chromosome 1D of bread wheat were also evaluated. In general, durum wheat cultivars with elevated gluten strength and/or increased dough extensibility were noted to have higher loaf volume (LV) than those with weaker gluten. The 1AS.1AL-1DL translocation line ‘L252’ carrying the LMW-1 banding pattern had better dough mixing stability and LV than the translocation lines with the LMW-2 banding pattern. The 1AS.1AL-1DL translocation lines had higher grain protein concentrations (GPC), but the lowest loaf volumes of all the lines tested. These translocation lines also exhibited unappealing external loaf quality (loaf shape and appearance) and poor internal loaf quality (crumb structure). Variation in bread-making quality attributes were observed among durum genotypes. ‘97Emmer19’ exhibited higher LV than all the durum wheats evaluated and approached the loaf volume achieved with the bread wheat cultivar ‘AC Superb’. Breeding lines derived from crosses of ‘97Emmer19’ to strong gluten durum cultivars (‘WB881’ or ‘AC Navigator’) had higher LV than those of the durum checks. ‘97Emmer19’ carried *Glu-A1a** (HMW-GS *1Ax1*) and the progeny carrying that allele generally exhibited higher loaf volumes. Durum wheat genotypes expressing the *Glu-B1d* (HMW-GS pair *Bx6+By8*) allele exhibited better overall bread-making quality compared with those expressing the *Glu-B1b* (HMW-GS pair *Bx7+By8*) allele. The durum cultivar ‘Arcola’ and the emmer-derived breeding line ‘2000EB4’, showed higher alveograph

extensibility (L) values than did the bread wheat check ‘AC Barrie’. The durum wheat genotypes (with the exception of ‘Stewart-63’) and emmer-derived breeding lines exhibited better dough extensibility than the USDA-ARS 1AS.1AL-1DL translocation lines. These results indicate that there is potential to select for genotypes with improved baking quality in durum breeding programs.

Acknowledgements

I express my sincere and profound gratitude to my supervisor Dr. Curtis Pozniak, for his belief in me, and constant guidance and encouragement throughout my M.Sc. program. His emphasis on quality and meaningful research afforded me an experience which will be applicable to my long-term career goals.

I would also like to thank my advisory committee, Dr. Pierre Hucl, Connie Briggs, Dr. Bob Tyler and Dr. Bruce Coulman, for their advice, encouragement and constructive suggestions. I would like to thank Dr. Alireza Navabi from the University of Guelph, Ontario, Canada for being my external examiner.

I am very grateful to Connie Briggs for her guidance and support with the laboratory experiments and for teaching me the milling and baking techniques. I would like to acknowledge the technical assistance of Pam Lynn from the wheat quality lab and Nizar Hirji and Vinh Tang from the durum wheat field lab. I would also like to express my greatest appreciation to Harv Braitenbach for helping me with the protein electrophoresis procedures.

I would like to thank Dr. Daryl L. Klindworth from the United States Department of Agriculture - Agricultural Research Service (USDA-ARS), Fargo, USA for providing the seeds of USDA-ARS 1AS.1AL-1DL translocation lines for this study. I also wish to thank Dallas Kessler from the Plant Genetic Resources of Canada (PGRC), Saskatoon for providing me with the 'Chinook' bread wheat sample.

Financial support for this project from the National Sciences and Engineering Research Council (NSERC) is gratefully acknowledged.

Finally, I am forever indebted to my parents, Purnachandra Rao Bandla and Anathalakshmi Bandla, for their understanding, endless patience and encouragement when it was most required.

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Abbreviations and Acronyms

AACC	American Association of Cereal Chemists
AAFC	Agriculture and Agri-Food Canada
AAFC-SPARC	Agriculture and Agri-Food Canada Semiarid Prairie Agricultural Research Centre
ANOVA	Analysis of Variance
A-PAGE	Acidic Polyacrylamide Gel Electrophoresis
a*	Flour/Semolina Redness
b*	Flour/Semolina Yellowness
BU	Brabender Units
°C	Degrees Celsius
CBB R-250	Coomassie Brilliant Blue R-250
CIE	International Commission on Illumination
CIMMYT	International Maize and Wheat Improvement Center
cc	cubic centimeters
cm	Centimeter
CSP	Canadian Short Process
CV	Coefficient of Variation
CWAD	Canadian Western Amber Durum
CWRS	Canadian Western Red Spring
d	days
DDT	Farinograph Dough Development Time
DG	Dry Gluten
DTT	Dithiothreitol
FAO	Food and Agriculture Organization of the United Nations
FAB	Farinograph Water Absorption
FN	Falling Number
FP/SP	Flour Protein/Semolina Protein
FY/SY	Flour Yield/Semolina Yield
g	gram
GDL	Goodale growing location
GI	Gluten Index
<i>Gli</i>	Gliadin
<i>Glu</i>	Glutenin
GMG	Methyl Green
GPC	Grain Protein Concentration
GRL	Grain Research Laboratory
GSPs	Grain Softness Proteins
<i>Gsp-D1</i>	Grain softness protein-1 gene
h	Hour
<i>Ha</i> locus	Grain Hardness locus
HI	Hardness Index
hL	Hectolitre
HMW	High Molecular Weight
HMW-GS	High Molecular Weight Glutenin Subunits

HMW-STD	High Molecular Weight Protein Standard
IMWG	Intermediate-molecular-weight group
KDa	Kilodalton
KER	Kernen growing location
Kg hL ⁻¹	Kilogram per hectolitre
KWT	Kernel Weight
<i>L</i>	Alveograph Extensibility
<i>L*</i>	Flour/semolina Brightness
LMW	Low Molecular Weight
LMW-GS	Low Molecular Weight Glutenin Subunits
LSM	Least Square Means
LSD	Least Significant Difference
LV	Loaf Volume
mb	Moisture Basis
mg kg ⁻¹	Milligrams per kilogram
min	Minute
mL	Millilitre
mm	Millimetre
mRNA	Messenger RiboNucleic Acid
MTI	Farinograph Mixing Tolerance Index
Nacl	Sodium chloride
nm	Nanometre
OD	Optical Density
<i>P</i>	Alveograph tenacity
<i>P/L</i>	Alveograph curve configuration ratio
<i>p^H</i>	Power of Hydrogen
<i>Pina-D1</i>	Puroindoline protein a
<i>Pinb-D1</i>	Puroindoline protein b
<i>Ph1</i>	Pairing Homoeologous gene
PMT	Peak Mixing Time
QTL	Quantitative Trait Loci
<i>r</i>	Correlation Coefficient
RCBD	Randomized Complete Block Design
RM	Relative Mobility
rpm	Revolutions Per Minute
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis
sec	seconds
SED	Standard Error of Difference
SF	Seed Farm growing location
SKCS	Single Kernel Characterization System
SK-HI	Single Kernel Hardness Index
STA	Farinograph Stability
TEMED	N,N,N,N-tetramethyl-ethylene diamine
TCA	Trichloroacetic acid

TTB	Farinograph Time to Breakdown
TWT	Test Weight
USDA-ARS	United States Department of Agriculture – Agricultural Research Service
<i>W</i>	Alveograph deformation energy
Wh/kg	Watt-hours per kilogram
WG	Wet Gluten
YPC	Yellow Pigment Concentration
α -gliadin	alpha gliadin
β -gliadin	beta gliadin
γ -gliadin	gamma gliadin
ω -gliadin	omega gliadin
μm	microns

1. Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is an important crop in Canada with 10-year (1993 - 2003) average annual production of 4.5 million tonnes (FAO, 2006). The majority of the durum wheat crop is produced in Saskatchewan, with 3.01 million tonnes produced in the 2007 crop year (Statistics Canada, 2008). Historically, durum production and consumption has been concentrated in the hot dry regions of North Africa, Southern Europe, Turkey and Syria, but production has expanded into North America, where a suitable climate is found in the major growing regions of western North Dakota and Montana in the United States, and southern Saskatchewan and Alberta in Canada (Market Analysis Division, AAFC, 2005). The trend worldwide is increased consumption of durum wheat products, including bread made from durum wheat semolina.

The poor bread-making quality of durum wheat has limited its wider use for bread production (Elias, 1995). The differences between common wheat and durum wheat can be attributed largely to their gluten protein properties, with durum wheat normally having weaker and less extensible gluten characteristics than bread wheat (Quaglia, 1988; Ammar et al., 2000; Edwards et al., 2001; Rao et al., 2001). However, the development of strong gluten durum cultivars has improved not only the cooking quality of pasta products, but has also resulted in improved bread-making quality (Liu et al., 1996; Dexter and Marchylo, 1996, 2000). Durum wheat baking quality does not appear to be linked to pasta cooking quality (Dexter and Marchylo, 2000), allowing for the development of durum wheat varieties suitable for both pasta-making and bread-making. The development of durum wheat with satisfactory bread baking characteristics and acceptable pasta quality would be beneficial, considering the potential benefit in the international market. Quick and Crawford (1983) reported that a dual-purpose durum wheat would have distinct advantages in situations where a processor could purchase one source of raw material for both bread flour and pasta semolina or when weather and disease caused a shortage of hexaploid wheat.

Although durum wheat is mostly used for pasta production, its use for the preparation of different kinds of bread is widespread in many Mediterranean countries

(Williams et al., 1984; Williams, 1985) and is increasing in North American countries (Faridi and Faubion, 1995; Dexter et al., 1998). Depending on the country and the amount of blending with other wheat flour, several types of bread are made from durum wheat (Elias, 1995). Only recently have investigations of durum wheat end-use for bread-making been undertaken. Hence, reported studies on durum wheat baking quality are few in number and only a limited number of genotypes have been investigated. The objective of this research was to develop a better understanding of the physical, chemical and rheological factors that influence the bread-making quality of durum wheat.

2. Literature Review

2.1. Durum Wheat and Its Uses

Durum wheat is an economically important cereal crop grown throughout the world, although not as extensively as bread wheat. Durum is grown on approximately 18 million hectares worldwide, with production averaging 30 million metric tonnes (International Grains Council, 2002; CFIA, 2006). The major durum producing countries are the European Union (Italy, Turkey, Spain, France, and Portugal), Canada, Syria, USA, Algeria and Morocco, while minor production areas include Russia, Tunisia, Mexico and India (Pasquale et al., 2007). Canada is the second largest producer (4.6 million metric tonnes per year), followed by Turkey (4 million metric tonnes) and the USA (3.5 million metric tonnes) (International Grains Council, 2002; CFIA, 2006). In Canada, durum wheat production occurs in the drier, south central regions of the prairie provinces of Manitoba (2% of Canadian production), Saskatchewan (84% of Canadian production) and Alberta (14% of Canadian production) (CFIA, 2006). In the Canadian wheat classification system, two sub-classes of durum wheat are recognized; conventional varieties with moderate gluten strength and extra-strong varieties with strong gluten properties similar to the USA desert durum varieties (Clarke et al., 2005).

Durum grain possesses unique quality characteristics (high yellow pigment content and hard vitreous kernels that will typically yield excellent quality semolina) that differentiate Canadian Western Amber Durum (CWAD) wheat from other Canadian wheat classes. Common or bread wheat is used for bread (leavened, flat, and steamed), noodles, biscuits, and cakes. In contrast, durum wheat is used predominantly for pasta and couscous (paste durum wheat products) and for bulgur and frekeh (non-paste durum wheat products) in the Middle East and North Africa. The use of durum wheat in flat and specialty breads is also common in Mediterranean countries, the Middle East, and North Africa (Quaglia, 1988; Boyacioglu and D'Appolonia, 1994; Boggini et al., 1995) and in recent years, its use in preparation of breads of all types is increasing (Palumbo et al., 2000). Durum wheat that combines pasta and baking quality (dual-purpose durum wheat) is a desirable goal as such cultivars would have alternative markets in years of high production, by being used in place of bread wheat, either alone or in blends with bread

wheat flour (Boggini and Pogna, 1989; Boggini et al., 1995). In bread wheat, the high molecular weight glutenin subunit pair (HMW-GS) *1Dx5+1Dy10* encoded by *Glu-D1d* allele is considered responsible for good bread-making quality, and in durum wheat, the low molecular weight glutenin subunits (LMW-GS) encoded by *Glu-B3* genes are responsible for good pasta cooking quality. Since different genes impart good pasta and bread-making qualities, it may be possible to improve the bread-making quality of durum wheat without negatively affecting pasta cooking quality (Marchylo et al., 2001).

2.2 Wheat Quality Factors

Wheat quality can be broadly defined into physical quality, chemical quality, and rheological and processing characteristics. Physical grain quality traits include kernel hardness, vitreousness of the grain, kernel weight, hectoliter weight, kernel size and shape, all of which can influence rheological and/or processing characteristics. Chemical quality traits include protein content and composition of gluten subunits and these two factors largely influence the rheological properties of dough including dough mixing characteristics and visco-elastic properties (Faridi, 1985; Bushuk, 1985; Menjivar, 1990; Kovacs et al., 1997; El-Khayat et al., 2006).

2.2.1 Physical Quality

2.2.1.1 Test Weight and Kernel Weight

Test weight is a measure of grain density, and is widely utilized as a wheat grading factor. Test weight is influenced by genetic factors and the environment with heritability estimates ranging from 0.44 to 0.83 (Bhatt and Derera, 1975). Grain that is badly shriveled as a result of disease or drought is usually low in test weight. Test weight has been suggested as a measure to predict milling potential, but there is no consensus on its true value as a milling yield predictor (Hook, 1984). Dexter et al. (1987) found a strong relationship between semolina yield and test weight for durum wheat. Watson et al. (1977) also concluded that test weight was an effective indicator of milling potential for durum wheat. However, different wheat classes and different varieties within a wheat class exhibit different relationships between test weight and milling yield (Dexter and Edwards, 1999). With lower test weights, the milling yield usually falls rapidly.

Thousand-kernel weight is a measure of average kernel size. Seed weight is under genetic control with quantitative trait loci (QTL) being reported on chromosomes 3D and

4A in hexaploid wheat (McCartney et al., 2005). There is also a strong environmental influence with heritability estimates in the range of 0.37 to 0.69 (Sharma and Knott, 1964; Jochum et al., 2001; Collaku and Harrison, 2005). Matsuo and Dexter (1980) reported a high correlation between milling yield and grain size in durum wheat. With large kernels a greater milling yield is generally expected due to a greater ratio of endosperm to bran. Larger kernels (kernel weight and volume) show a negative association with protein and gluten content (Khattak et al., 2005).

2.2.1.2 Kernel Hardness

Kernel hardness is an important factor in determining the end-use suitability of wheat. Soft wheats are more friable, require less energy to mill and produce flours and whole wheat meals with reduced particle-size, including many free starch granules (Cutler and Brinson, 1935; Devaux et al., 1998) which makes soft wheats useful for cookies, cakes, and pastries (Peña, 1997). In contrast, the protein and starch matrices are tightly bound in the endosperm of hard wheats (Donelson and Yamazaki, 1962). Hardness of the grain affects the manner in which the endosperm and starch granules are fractured during the milling process, and hard wheat flours generally have more broken and damaged starch granules. Damaged starch absorbs more water than intact starch granules during dough mixing (Evans and Stevens, 1985), thus hard wheat flours exhibit increased farinograph water absorption (FAB) (Tipples, 1969; Bass, 1988).

In hexaploid wheat, the genetic control of kernel hardness is well understood, and is largely controlled by the action of two tightly linked puroindoline a (*Pina-D1*) and puroindoline b (*Pinb-D1*) genes at the Hardness (*Ha*) locus on the distal end of the short arm of chromosome 5D (Bhave and Morris, 2007). Soft texture is the wild type (*Pina-D1a/Pinb-D1a*) with hard texture being determined by mutations in either *Pina-D1* or *Pinb-D1*. Absence of *Pina-D1* and *Pinb-D1* mRNA transcripts in durum wheat are consistent with its lack of the hexaploid wheat D genome, and results in very hard endosperm texture (Giroux et al., 2000). While the *Pina-D1* and *Pinb-D1* genes are major genetic factors responsible for grain hardness, they do not appear to account for the differences in wheat grain texture among wheat varieties or within crosses of the same textural class (Pickering and Bhave, 2007). Grain softness proteins (GSPs) are closely related to puroindolines (Gautier et al., 2000) and the grain softness protein-1 gene (*Gsp-*

DI) is closely linked to the *Ha* locus in wheat (Jolly et al., 1996) and einkorn wheat (*T. monococcum* L.) (*Gsp-A^m1*) (Chantret et al., 2004), where *Pina-DI* is positioned between *Pinb-DI* and *Gsp-1* (Tranquilli et al., 1999; Turnbull et al., 2003). Despite the close relationship among these three genes, there is no clear evidence of the role of the *Gsp-1* gene in grain kernel hardness (Tranquilli et al., 2002).

2.2.2 Protein Quality and Quantity

The ability of wheat flour to be processed into different products is largely determined by the gluten proteins (Weegels et al., 1996). The gluten proteins have been studied intensively to determine their structural properties and to provide a basis for manipulating and improving end-use quality (Shewry et al., 1995). Wheat grain proteins can be classified on the basis of their solubility in different solvents: albumins (soluble in water), globulins (salt), and prolamins [gliadins (alcohol) and glutenins (dilute acid or alkali)] (Mifflin et al., 1983; Shewry et al., 1986). The largest portion of the wheat storage proteins are the prolamins which are characterized by further repeated regions, rich in proline and glutamine. Wheat prolamins have been classified into two groups, the gliadins and glutenins, according to their solubility in aqueous/alcohol solutions (Kasarda, 1989; Shewry and Tatham, 1990). Gliadins are a mixture of monomeric polypeptides (Sapirstein and Fu, 1998) and glutenins consist of polypeptides aggregated by disulphide bonds (Shewry and Tatham, 1990; Singh and MacRitchie, 2001). The gliadins and glutenins constitute up to 80 to 85% of the total flour protein, and confer elasticity and extensibility properties that are essential for functionality of wheat flours (Branlard and Dardevet, 1985a, b; Shewry et al., 1995).

2.2.2.1 Albumins and Globulins

Water-soluble albumins and salt-soluble globulins constitute from 10 to 22% of the total flour protein (Singh and MacRitchie, 2001). Albumins such as α -amylase/trypsin inhibitors (Shewry et al., 1984; Buonocore et al., 1985), serpins (Østergaard et al., 2000) and purotionins (Garcia- Olmedo et al., 2002) may have dual roles as nutrient reserves for the germinating embryo and as inhibitors of insects and fungal pathogens prior to germination. The puroindolines are included in this group and influence grain hardness (see section 2.2.1.2). Generally, albumins and globulins do not have a direct impact on dough rheology, although a minor influence on bread-making quality has been reported

(Schofield and Booth, 1983).

2.2.2.2 *Glutenins*

The glutenins are polymeric proteins stabilized by disulfide bonds (Kasarda, 1989) that, when treated with a reducing agent, release high molecular weight glutenin subunits (HMW-GS; 90 to 140 KDa) and low molecular weight glutenin subunits (LMW-GS; 30 to 75 KDa). The HMW-GS and LMW-GS are considered the major factors that determine the visco-elastic properties of gluten (Payne et al., 1984; Klindworth et al., 2005). The HMW-GS are minor components in terms of quantity (5-10% of total protein; Payne, 1987), but they are key factors in the process of bread-making because they are major determinants of gluten elasticity (Tatham et al., 1985) allowing efficient trapping of gas for dough to rise (Cornish et al., 2006). The HMW-GS are encoded by genes at three loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, located on the long arms of homoeologous group 1 chromosomes (Payne et al., 1981; Payne and Lawrence, 1983). Molecular studies have shown that each locus contains two tightly linked genes which encode two types of HMW-GS, one of higher molecular weight, designated the x-type, and the other of lower molecular weight, designated the y-type (Harberd et al., 1986). Alleles coding for different subunits occur at all three loci (Lawrence and Shepherd, 1981; Payne et al., 1981) and are manifested as one or more subunit combinations, resulting in a high degree of subunit polymorphism in both bread and durum wheat cultivars (Payne and Lawrence, 1983; Branlard et al., 1989). The polymorphisms of glutenin coding alleles have been well described (Payne and Lawrence, 1983; Payne et al., 1987; Rogers et al., 1989; Gupta and Shepherd, 1990; Carillo et al., 1990; Metakovsky, 1991) and these are known to account for a part of the range in bread-making ability and pasta quality, depending on the fraction involved (Gupta et al., 1989; Khelifi and Branlard, 1992; Nieto-Taladriz et al., 1994). Glutenin proteins are responsible in part for the quality differences between durum and bread wheat (Vazquez et al., 1996).

Numerous studies have defined the molecular basis of bread-making and pasta quality in relation to specific polypeptides of the gluten protein complex, especially the HMW-GS (Payne et al., 1984; MacRitchie et al., 1990). Working with near-isogenic lines of common wheat, Redaelli et al. (1997) established that allelic variation at the *Glu-D1* locus had a greater influence on bread-making quality than the variation at the *Glu-A1*

and *Glu-B1* loci. In bread wheat, the HMW-GS *1Dx5 + 1Dy10* encoded by the *Glu-D1d* locus are associated with good bread-making quality and increased dough strength, while *1Dx2 + 1Dy12* encoded by *Glu-D1a* are associated with poor bread-making quality and weak dough (Payne et al., 1984, 1987, Shewry et al., 1992). As a result, *Glu-D1d* predominates in high-quality wheats, whereas allele *Glu-D1a* usually occurs in feed wheats with low bread-making quality (Rogers et al.; 1989; Groeger et al., 1997). The superior quality of the *Glu-D1d* allele is generally attributed to the difference in amino acid primary structures of *1Dx2* and *1Dx5*. According to Shewry et al. (1997), *1Dx5* has one additional cysteine residue and therefore can form longer polymer chains, resulting in higher elasticity of the dough.

The low molecular weight glutenin subunits (LMW-GS) represent approximately 40% of the total wheat gluten fraction (Payne, 1987; Gupta et al., 1989; Ciaffi et al., 1999), and most closely resemble γ -gliadins in sequence (Muller et al., 1998). The LMW-GS are encoded by genes at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci on the short arms of chromosomes 1A, 1B and 1D, respectively (Gupta and Shepherd, 1990). Features of these proteins, such as the distribution of the cysteine residues available for intermolecular disulphide bonds (Shewry and Tatham, 1997), as well as their overall amino acid compositions involved in noncovalent bonds (Bloksma and Bushuk, 1988; Pomeranz, 1988), are important in determining the rheological properties of dough. The LMW-GS function as chain terminators or extenders according to the number of cysteine residues available for disulfide bonding (Greenfield et al., 1998). Those LMW-GS associated with branch extension, in conjunction with HMW-GS, are thought to increase the polymer size and confer dough strength (Pogna et al., 1996; Lafiandra et al., 1999) and largely influence dough-mixing time (Gupta and Shepherd, 1988; Gupta et al., 1989; Pogna et al., 1990; Gupta et al., 1991; Nieto-Taladriz et al., 1994; Sissons et al., 1998).

As LMW-GS are present in greater amounts than HMW-GS, effort has been made to establish their role in bread-making quality (Payne, 1987; Gupta and Shepherd, 1987, 1988; Boggini and Pogna, 1989; Gupta et al., 1989; Metakovsky et al., 1990; Pogna et al., 1990). The LMW-GS are less well characterized than the HMW-GS, because large numbers of the LMW-GS subunits with similar mobility in SDS-PAGE analysis makes characterization difficult (D'Ovidio and Masci, 2004). In general, the LMW-GS are

associated with dough resistance and extensibility (Metakovsky et al., 1990; Andrews et al., 1994; Cornish et al., 2001), and some allelic forms show greater effects on these properties than HMW-GS (Payne et al., 1987; Gupta et al., 1989, 1994). The LMW-GS gene family has been studied in related wild wheat species and in several common wheat and durum wheat cultivars (Ciaffi et al., 1999; Lee et al., 1999; Masci et al., 2000; Ikeda et al., 2002; Wicker et al., 2003). Differences in the expression of LMW-GS, associated with specific allelic forms, have been reported to be important in conferring quality differences in bread wheat (Gupta and Shepherd, 1990). Boggini and Pogna (1989) have confirmed that γ -gliadin 45 has a strongly favorable influence on the bread-making quality of durum wheat as well. Redaelli et al. (1997) have shown strong positive effects on dough extensibility by *Gli-D1* (gliadins)/*Glu-D3* (LMW-GS) alleles.

In durum wheat, the LMW-GS encoded by the *Glu-B3* genes on chromosome 1BS (Joppa et al., 1983) are most responsible for good pasta quality (Ciaffi et al., 1991; Brites and Carrillio, 2001). Two LMW-GS patterns, LMW-1 and LMW-2, explain a large part of the quality differences between some durum wheat genotypes where the presence of LMW-2 glutenin subunits confers stronger gluten and better pasta-making quality than cultivars possessing LMW-1 (D'Ovidio, 1993; Vazquez et al., 1996). Durum wheat cultivars with LMW-2 have a greater amount of LMW-GS than LMW-1 type durum wheats (Autran et al., 1987; Masci et al., 1995). The *Glu-B3* locus is tightly linked to the *Gli-B1* loci that encode γ and ω - gliadins (Brown and Flavell, 1981) (see section 2.2.2.3). The LMW-1 group is linked to γ -gliadin 42 and to the three ω -gliadin subunits 33, 35, and 38, whereas the LMW-2 group is linked to γ -gliadin 45 and ω -gliadin 35, and these gliadins have been used as effective genetic markers for LMW-1 and LMW-2 (Payne et al., 1984, 1987).

At present, most of the durum wheat breeding programs have fixed the LMW-2/ γ -45/ ω -gliadin 35 loci because of their positive effect on pasta performance. However, large differences in pasta quality are still evident, suggesting other factors like the negative influence of the intermediate-molecular-weight group (IMWG) (albumins, globulins, some glutenins and omega gliadins) on pasta quality (Galterio et al., 1993).

2.2.2.3 Gliadins

The gliadins are divided into four groups, alpha- (α -), beta- (β -), gamma- (γ -), and

omega- (ω -) gliadins, based on their electrophoretic mobility at low pH (Woychik et al., 1961). The amino acid compositions of the α - , β - , γ - and ω - gliadins are similar (Tatham et al., 1990), although the ω -gliadins contain little or no cysteine or methionine residues and only small amounts of glutamine, proline and phenylalanine (Tatham and Shewry, 1995). Thus, all gliadins are monomers with either no disulphide bonds (ω -gliadins) or intra-chain disulphide bonds (α -, β - and γ - gliadins) (Muller and Wieser, 1995, 1997). Gliadins are encoded by six *Gli* loci mapped to the short arms of homoeologous group 1 (*Gli-A1*, *Gli-B1* and *Gli-D1*) and 6 (*Gli-A2*, *Gli-B2* and *Gli-D2*) chromosomes (Wrigley and Shepherd, 1973; Payne et al., 1982). The significance of gliadin subgroups in the functionality of wheat flour has been debated in the literature. In durum wheat, a highly significant correlation between specific γ -gliadin components and gluten visco-elasticity has been demonstrated (Damidaux et al., 1978; Kosmolak et al., 1980). However, as indicated in section 2.2.2.2, the γ -42 and γ -45 gliadins are allelic variants whose coding genes at the *Gli-B1* locus (Joppa et al., 1983) are linked to genes at the *Glu-B3* locus (Gupta and Shepherd, 1987), making it difficult to determine the individual effects of the γ -gliadins.

There exist several references on the relationship of gliadin alleles to dough quality (Sozinov and Popereya, 1982; Wrigley et al., 1981; Pogna et al., 1982; Dachkevitch et al., 1993). In dough formation, the gliadins do not become covalently-linked into large elastic networks, but act as a ‘plasticiser’, promoting viscous flow and extensibility which are important rheological characteristics of dough. Gliadins have been postulated to interact with other proteins through a disulphide interchanging, and through hydrophobic and hydrogen bonding (Beitz and Wall, 1980; Khatkar et al., 2002). In addition, the ratio of monomeric gliadins to polymeric glutenin proteins (Gupta et al., 1992; Sapirstein and Fu, 1998) and the amount and size distribution of polymeric proteins (Gupta et al., 1993; MacRitchie, 1999; Johansson et al., 2001) determine protein quality. Fu and Sapirstein (1996) confirmed that most of the variation in dough strength parameters was explained by the relative proportions of soluble and insoluble glutenins.

2.2.3 Durum Wheat Quality

2.2.3.1 Semolina milling

The process of wheat milling separates the starchy endosperm (83% of wheat

kernel) from the bran (pericarp, testa, aleurone, nucellus, some starch endosperm) (14.5% of wheat kernel) and the embryo (germ) (2.5% of wheat kernel). The separation should ideally occur at the level of the endosperm/aleurone layer interface if aleurone, which is high in ash content, is to be excluded from the semolina. According to Chaurand et al. (1999), semolina milling potential depends on three main factors: external factors related to growing and harvesting conditions; internal factors such as the endosperm/bran ratio and the mechanical resistance or friability of the endosperm (semolina/flour ratio); and the ease of separating the endosperm from the bran, which is a function of kernel hardness. Semolina milling yield is highly dependent on the cultivar and the agronomic conditions within which the cultivar was grown (Clarke et al., 1998; Troccoli et al., 2000). Commercial semolina extraction rates range from 65 to 72% (Matz, 1991; Blazek et al., 2005; Hruskova et al., 2006). Semolina purification remains the most important process of durum wheat milling (Dexter and Marchylo, 2000).

The hardness of starchy endosperm has been the subject of many studies and is identified as a major factor influencing durum wheat semolina milling behavior (Lempereur et al., 1997; Chaurand et al., 1999). The physical characteristics of durum wheat, such as test weight, kernel weight, kernel size and degree of vitreousness have also been known to influence the milling performance of durum wheat (Dexter et al., 1987; Dexter et al., 1988; Troccoli et al., 2000). Grain conditioning/tempering induces an increase in bran extensibility, while preserving the hardness of the starchy endosperm (Peyron et al., 2002), and is a common practice prior to milling.

2.2.3.2 Semolina and pasta colour

The yellow colour of the semolina and pasta is an important end-use quality trait in the international market (Troccoli et al., 2000; Marchylo et al., 2001). The colour of semolina and pasta products is often expressed using CIELab colour scale [L* (brightness) a* (redness) b* (yellowness)]. The bright yellow colour of durum pasta is a function of the concentration of carotenoid pigments, mainly lutein in free ester form, present in the grain (Irvine and Anderson, 1953; Laignelet, 1983; Mann et al., 1998; Borrelli et al., 1999). However, Hentschel et al. (2002), applying more sophisticated separation techniques, found that the chemical nature of the yellow pigment in semolina is quite complex and concluded that the carotenoids fraction accounted for only 30-50%

of the total yellow pigment, while the rest were unidentified compounds. However, the authors did not determine whether these unknown compounds are forms of lutein modified during the extraction process.

The genetics of yellow pigment are well understood in durum wheat. The trait is highly heritable (Elouafi et al., 2001; Clarke et al., 2006) and quantitative trait loci (QTL) have been identified on most chromosomes. However, a QTL with a large effect on yellow pigment in the distal region of the long arm of chromosome 7B has been identified in numerous mapping populations (Elouafi et al., 2001; Pozniak et al., 2007). A QTL with a smaller effect has also been reported in the distal region of chromosome arm 7AL (Elouafi et al., 2001). A gene coding for phytoene synthase, the first enzyme involved in the carotenoid biosynthesis pathway, was shown to co-segregate with the 7B QTL, and has been suggested as a gene to be targeted for marker assisted selection to enhance yellow pigment (Pozniak et al., 2007). Additional QTL have been identified on chromosomes 5A (Hessler et al., 2002), 1B and 6A (Zhang et al., 2005), and chromosomes 2A, 4B, and 6B (Pozniak et al., 2007).

Pasta colour loss during processing is common and is largely the result of LOX (EC 1.13.11.12) enzyme activity (Borrelli et al., 1999). Lipoxygenases catalyze the breakdown of polyunsaturated fatty acids in plants (Prigge et al., 1996), and in durum wheat are responsible for degradation of the yellow colour in pasta by oxidation (Joppa and Williams, 1988b). Carotenoid pigment degradation is particularly high at the beginning of dough mixing when oxygen and lipid, the primary substrates of LOX, are present in the highest amounts and the mixing enhances the incorporation of oxygen in the dough (Delcros et al., 1998; Rakotozafy et al., 1999).

Using nulli-tetrasomic lines, Hart and Langston (1977) assigned wheat LOX isoenzymes to chromosomes 4A (*Lpx-A1*), 4D (*Lpx-D1*), 5A (*Lpx-A2*), 5B (*Lpx-B2*) and 5D (*Lpx-D2*). Two linked copies of *Lpx-B1*, *Lpx-B1.1* and *Lpx-B1.2*, (van Mechelen et al., 1999; Ramakrishna et al., 2002) exist on 4B. The *Lpx-B1.1* locus was concluded to be a valuable breeding target for durum wheat breeding programs aimed at improving pasta colour as that locus alone explained 54% of the variation in LOX activity ($P < 0.0001$) (Carrera et al., 2007). Although other enzymes, such as peroxidases and polyphenol oxidases, contribute to semolina bleaching (Taha and Sagi, 1987), LOX plays a major

role, which catalyses the hydro-peroxidation of the polyunsaturated fattyacids containing 1,4-cis, cis pentadiene structures (Gradner, 1988; Siedow, 1991); in particular, free linoleate in durum wheat semolina is oxidised (Matsuo et al., 1970), thus causing semolina bleaching (Trono et al., 1999). Hence, reducing lipoxygenase activity in varieties possessing other high quality attributes is highly desirable to maintain yellow pasta colour.

2.2.3.3 Alpha-amylase enzyme activity and falling number

Alpha-amylase activity in pre-harvest sprouted wheat grain results in the degradation of starch into simple sugars (Kruger, 1972). Screening for resistance to pre-harvest sprouting is a high priority in wheat breeding programs, because the majority of end products are negatively influenced by this enzyme. Several methods exist to evaluate the effects of α -amylase (Hagberg, 1960). The Falling Number (FN) method is widely used commercially and in breeding programs. Although the FN test does not measure α -amylase enzyme levels directly, it measures changes in the physical properties of starch that result from α -amylase activity and is sufficiently accurate for most quality tasks (Blackman and Payne, 1987; Every et al., 2002; Lessard, 2002). The amylograph (C.W. Brabender Instruments, Inc.) and the Rapid Visco Analyser (Newport Scientific, Warriewood, Australia) can also be used to assess starch properties and the negative effects of α -amylase on starch (Atwell, 2001).

Pre-harvest sprouting can affect the end product made from wheat in many ways (Roozeboom et al., 1999). Increased levels of amylases in wheat have deleterious effects on processing quality and on the bread-making quality of flour and dough (Lessard, 2002). Alpha-amylase hydrolyses starch during mixing and fermentation, reducing the water holding capacity of starch and lowering baking absorption. This results in a sticky dough that is difficult to handle (Blackman and Payne, 1987; Dexter and Edwards, 1999). Studies (Matsuo et al., 1982; Dick et al., 1974) have found that sprout damage has little effect on pasta texture. Selection for wheat lines with low levels of resistance to premature germination is difficult because of the large environmental influence on expression of sprouting and α -amylase production (Derera et al., 1977; Bassoi et al., 2006), but progress has been made in characterization of QTL controlling genetic variation for pre-harvest sprouting (Zanetti et al., 2000; Imtiaz et al., 2008).

2.2.3.4 Pasta cooking quality of durum wheat

Breeding for pasta quality is a major objective of Canadian durum breeding programs (Clarke et al., 1998). High quality durum pasta maintains a firm texture when cooked, and its natural amber colour is associated with good quality pasta. Durum protein quantity and gluten quality have an important effect on pasta-making characteristics and resistance to overcooking. Pasta-making quality generally is measured in terms of pasta stickiness, firmness, cooking tolerance and water absorption (Pogna et al., 1994). A number of workers have developed successful methods for estimating cooked spaghetti firmness and resilience (e.g., Dexter et al., 1985) and have associated cooking quality with protein content, gluten composition and solubility, farinograph mixing characteristics, SDS-sedimentation volume and mixograph characteristics (Matsuo et al., 1982). Kovacs et al. (1997) showed that pasta disc viscoelasticity, mixograph parameters such as peak height and total energy, and alveograph curve parameters were highly correlated with chewiness and firmness, as determined by sensory analysis.

High protein content or, rather, a high content of all nitrogenous substances (Dexter and Marchylo, 1996; Clarke et al., 1998) as well as high gluten strength and elasticity (Clarke et al., 1998; Edwards et al., 2001; Bechere et al., 2002) influence pasta resistance to overcooking. Pasta resistance to overcooking is also influenced by pasta drying temperature (Guler et al., 2002; Villeneuve and Gelinias, 2007).

2.2.3.5 Bread baking quality of durum wheat

Genetically, durum wheats are tetraploids (AABB), and lack the D genome found in hexaploid (AABBDD) bread wheats. Removal of the D genome from hexaploid bread wheat greatly reduces its baking potential (Kerber and Tipples, 1969) and is considered at least partly responsible for the relatively poor baking quality of durum wheat (Joppa and Williams, 1988a). Quaglia (1988) identified inextensible dough characteristics as the major factor limiting loaf volume potential of strong Italian durum wheat cultivars. Ammar et al. (2000) suggested that inadequate dough extensibility, i.e., lower alveograph extensibility (L) and greater tenacity to extensibility ratio (P/L), prevents durum wheat from achieving loaf volumes equivalent to those of bread wheat. The baking performance of durum wheat increases as gluten becomes stronger (Quick and Crawford, 1983; Josephides et al., 1987; Boggini and Pogna, 1989; Lopez-Ahumada et al., 1991; Dexter et

al., 1981, 1994; Boyacioglu and D'Appolonia, 1994; Peña et al., 1994; Boggini et al., 1995; Hareland and Pühr, 1999; Sapirstein et al., 2007; Edwards et al., 2007). However, very strong gluten durum wheat has a tendency to exhibit tenacious gluten, imparting inextensible dough and lower loaf volume due to reduced oven response (Quaglia, 1988; Ammar et al., 2000; Edwards et al., 2001; Rao et al., 2001). Together, these results suggest that to develop durum wheat cultivars with loaf volumes (LV) approaching that of bread wheat, it may be necessary to achieve an appropriate balance of resistance to extension (i.e., alveograph *P* or tenacity) and extensibility (alveograph *L*) in conjunction with increased alveograph *W* values or overall strength (Dexter et al., 1994).

There is general agreement that durum wheat baking performance improves as gluten becomes stronger, but remains inferior to bread wheat. However, the type of baking process used to evaluate baking potential will influence the relative differences in baking performance among durum cultivars (Dexter and Marchylo, 2000; Sapirstein et al., 2007; Edwards et al., 2007). Longer fermentation baking methods, such as the remix-to-peak process and the sponge-and-dough procedure, are commonly used to assess durum wheat baking potential (Dexter et al. 1998). When baked by the remix-to-peak process, weaker durum cultivars show reduced loaf volume compared to stronger cultivars and stronger durum wheat genotypes exhibit bread attributes and loaf volume comparable to what would be expected from a standard bread wheat variety (Rao et al., 2001; Sapirstein et al., 2007). In contrast, Dexter et al. (1994) and Marchylo et al. (2001) showed that Canadian durum wheat of moderate strength produced good bread when baked by a short process, whereas baking quality of moderate strength durum wheat was poor when using long fermentation times. Sapirstein et al. (2007) further confirmed that the short fermentation time (when reduced from the standard 165 to 90 min to 15 min) is advantageous for durum wheat bread quality, likely because durum wheat genotypes tend to possess inferior fermentation tolerance compared to the bread wheat flour (Edwards et al., 2007). A factor associated with the lack of fermentation tolerance in durum wheats is the absence of HMW-GS encoded by *Glu-D1* loci (Sapirstein et al., 2007). The lack of fermentation tolerance could also be attributed to the degree of protein quality in durum wheat, although there is no literature published on the relationship between lack of fermentation tolerance and protein quality in durum wheat.

Transfer of the *Glu-D1d* alleles coding for *1Dx5+1Dy10* (responsible for good baking quality) from common wheat to durum wheat has been examined as a means to enhance the bread-making quality of durum wheat (Pogna et al., 1996; Ceoloni et al., 1996; Joppa et al., 1998; Lafiandra et al., 2000). Joppa et al. (1998) successfully transferred the segment of chromosome 1D coding for *1Dx5+1Dy10* from hexaploid wheat cultivar ‘Len’ to durum wheat cultivars ‘Renville’ and ‘Langdon’. Detailed cytogenetic and molecular studies have confirmed that the 1DL substitution spans approximately 31% of the long arm of the 1AS chromosome (Xu et al., 2005). Blanco et al., (2002) and Lukaszewski, (2003) backcrossed a 1AS.1DL translocation from triticale (\times *Triticosecale* Wittmack) into durum genetic backgrounds. Vitellozzi et al. (1997) also produced a durum 1AS.1AL-1DL translocation, induced through *ph1*-mediated homoeologous pairing. Preliminary bake tests conducted by Joppa et al. (1998) suggested that the 1AS.1DL translocation lines had improved loaf volumes compared to near isogenic lines lacking the translocation. However, detailed studies conducted by Klindworth et al. (2005) have shown that the 1AS.1DL translocation did not significantly improve loaf volume compared to its isogenic parent “Renville”. Also, they did note that translocation lines carrying LMW-1 had better mixing and baking characteristics than lines carrying the strong gluten LMW-2 banding patterns (Klindworth et al., 2005). In contrast, Liu et al. (1995) reported improvement in baking quality in ‘Langdon’ substitution lines carrying the *Glu-D1a* allele. ‘Langdon’ carries the LMW-1 glutenin subunit pattern (Joppa et al., 1998).

Another possibility for improving the bread-making quality of durum wheat is to identify wild relatives that exhibit variation for enhanced baking quality. Emmer wheat (*Triticum turgidum* var. *dicoccum*), a tetraploid (AABB) wheat, is the evolutionary precursor to durum wheat. Numerous studies have examined the potential for emmer wheat as a source of genetic variation to improve the baking quality of durum wheat. Baking studies conducted as early as 1918 indicated that some emmer lines had baking qualities superior to that observed in durum wheat cultivars (LeClerc et al., 1918). Peña et al. (1993) evaluated approximately 150 emmer accessions using gel electrophoresis and identified some accessions with glutenin subunits known to contribute to enhanced bread quality. Similarly, Blum et al. (1984) evaluated over 800 wild emmer lines,

resulting in the identification of lines which had high protein and high molecular weight subunits associated with good bread baking quality. Schlichting et al. (2002) reported the identification of Emmer wheat ‘97Emmer19’ which displayed improved baking quality over several Canadian durum wheat cultivars. Preliminary evaluation of breeding lines derived from crossing ‘97Emmer19’ to strong durum wheat cultivars indicated that most lines had improved baking quality while retaining the good pasta cooking quality of the durum wheat parents (Schlichting et al., 2002). Furthermore, ‘97Emmer19’ has since been crossed to adapted durum wheat cultivars, and there is a need for further research to evaluate the potential of ‘97Emmer19’-durum derivatives for enhanced baking quality and to characterize factors that are contributing to improved baking quality.

2.3 Research Hypothesis

1. ‘97Emmer19’ has shown improved baking quality relative to commercial durum wheat cultivars. This research was designed to test the hypothesis that ‘97Emmer19’ contains factors contributing to physical, chemical and rheological properties that enhance baking quality, and in particular improved dough extensibility. Progeny from crosses of ‘97Emmer19’ to strong gluten durum cultivars were included to test the hypothesis that these factors were heritable.
2. Variation at gliadin and glutenin subunits is known to influence bread-making quality of wheat. An additional hypothesis of this research is that ‘97Emmer19’ possesses unique gliadin and glutenin subunits that contribute to its enhanced baking quality. If this hypothesis is true, progeny from ‘97Emmer19’ with similar subunit composition should display enhanced baking quality over commercial durum wheat cultivars.

2.4 Objectives

The objectives of this study were:

1. To determine the inter-relationships between physical quality traits, chemical quality traits and rheological properties and enhanced bread-making quality of durum and ‘97Emmer19’-derived genotypes.

2. To identify gliadins and glutenins from '97Emmer19' and other emmer derived lines which may be associated with enhanced baking quality.

3. Materials and Methods

3.1 Plant Material and Experimental Design

Twenty-nine genotypes (Table 1) were used in this study. ‘97Emmer19’, a hulled Emmer wheat, with good baking quality (Schlichting et al., 2002) was evaluated along with seven durum wheat varieties and breeding lines developed by crossing ‘97Emmer19’ with strong durum cultivars (‘WB881’ or ‘AC Navigator’). Three Canadian Western Red Spring (CWRS) varieties ‘CDC Teal’, ‘AC Barrie’, and ‘AC Superb’, which represent the baking quality of current bread wheat varieties grown in Canada, were included as positive controls and bread-making standards. ‘Commander’ and ‘WB881’ are semi-dwarf durum cultivars with extra-strong gluten properties. ‘DT724’, developed by Agriculture and Agri-Food Canada Semiarid Prairie Agricultural Research Centre (AAFC-SPARC) also possesses extra-strong gluten. ‘Strongfield’ and ‘AC Navigator’ possess moderate gluten strength. ‘Arcola’ was included in this study as it possesses the HMW-GS pair $Bx7+By8$ (Ng et al., 1988b). ‘L092’, ‘L252’, ‘S99B33’, and ‘S99B34’ are 1AS.1AL-1DL translocation lines and were obtained from United States Department of Agriculture - Agricultural Research Service (USDA-ARS), North Dakota. ‘04EDUYT-42’, ‘04EDUYT-43’, ‘04IDSN-107’, and ‘04IDSN-111’ lines were obtained from the International Maize and Wheat Improvement Center (CIMMYT), Mexico and at the time of evaluation these lines were believed to carry 1AS.1AL-1DL translocation. Twenty-five genotypes were tested in 2005 growing season and twenty-four genotypes in 2006 (Table 1). Genotypes were grown in 1.2 m x 3.6 m plots arranged in a randomized complete block design (RCBD) with three replicates at each of three locations [Kernen (KER), Goodale (GDL), and Seed Farm (SF); Saskatoon] in 2005 and were repeated at the same testing sites in 2006 growing season. Seeding rates were 250 seeds per m² plot with a spacing of 20 cm between rows. Plots were sown on 3 May (GDL), 14 May (KER), and 6 May (SF) in 2005 and 18 May (GDL), 16 May (KER), and 2 May (SF) in 2006. Data from ‘S99B33’ was lost from SF location for 2006 growing season because of a seeding error. At maturity, plots were harvested with a small plot combine and samples were dried to approx. 9% moisture.

Table 1. Pedigree classification and country of origin of the bread wheat checks (AC Barrie, CDC Teal, and AC Superb) and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines) used for this study.

Genotype	Pedigree	Source/Origin	2005 ^a	2006 ^b
BREAD WHEAT CHECK CULTIVARS				
AC Barrie	Neepawa/Columbus//BW90	Canada	Grown	Grown
CDC Teal	BW514/Benito//BW38	Canada	Grown	Grown
AC Superb	Grandin2*/AC Domain	Canada	Grown	Grown
1AS.1AL-1DL TRANSLOCATION LINES				
04EDUYT-42	1A.1D 5+10-6/2*WB881//1A.1D 5+10-6/3*Mojo/Bisu_1/Patka	CIMMYT ^c - Mexico	Grown	-
04EDUYT-43	1A.1D 5+10-6/2*WB881//1A.1D 5+10-6/3*Mojo/Bisu_1/Patka	CIMMYT- Mexico	Grown	-
04IDSN-107	1A.1D 5+10-6/2*WB881//1A.1D 5+10-6/3*Mojo/Bisu_1/Patka	CIMMYT- Mexico	Grown	-
04IDSN-111	1A.1D 5+10-6/2*WB881//1A.1D 5+10-6/3*Mojo/Bisu_1/Patka	CIMMYT- Mexico	Grown	-
L092	1A.1D/Len//Langdon/3/2*Renville	USDA-ARS ^d North Dakota	Grown	Grown
L252	1A.1D/Len//Langdon/3/2*Renville	USDA-ARS North Dakota	Grown	Grown
S99B33	1A.1D/Len//Langdon/3/2*Renville	USDA-ARS North Dakota	Grown	Grown
S99B34	1A.1D/Len//Langdon/3/2*Renville	USDA-ARS North Dakota	Grown	Grown
DURUM WHEAT CULTIVARS				
Strongfield	AC Avonlea//Kyle/Nile	Canada	Grown	Grown
WB881	PI 483458	Iran	Grown	Grown
Commander	W9260-BK03/AC Navigator//AC Pathfinder	Canada	Grown	Grown
AC Navigator	Kyle/Westbred 881	Canada	Grown	Grown
DT724	DT666/DT665	Canada	Grown	Grown
Stewart-63	ST464/8*Stewart	Canada	Grown	Grown
Arcola	Wascana/Hercules	Canada	Grown	Grown
‘97EMMER19’ AND EMMER-DERIVED BREEDING LINES				
97Emmer19	PI 195721	Iran	Grown	Grown
2000EB4	WB881/97Emmer19	Canada	Grown	Grown
X.98.142.17	WB881*2/97Emmer19	Canada	Grown	Grown
X.98.142.18	WB881*2/97Emmer19	Canada	Grown	-
P.01.64.31	AC Navigator//2000EB4/AC Navigator	Canada	Grown	Grown
P.01.64.39	AC Navigator//2000EB4/AC Navigator	Canada	Grown	Grown
P.01.64.62	AC Navigator//2000EB4/AC Navigator	Canada	Grown	Grown
05Emmereg-01	2000EB4/AC Navigator	Canada	-	Grown
05Emmereg-03	2000EB4/AC Avonlea	Canada	-	Grown
05Emmereg-10	2000EB4/AC Avonlea	Canada	-	Grown
05Emmereg-26	2000EB4/AC Navigator	Canada	-	Grown

^a2005 = Genotypes grown in 2005 growing season ^b2006 = Genotypes grown in 2006 growing season ^cCIMMYT = International Maize and Wheat Improvement Center ^dUSDA-ARS = United States Department of Agriculture – Agricultural Research Service

3.2 Quality Evaluation of Non-composite Samples (Whole Grain Quality Measures)

3.2.1 Physical Grain Quality

Following harvest, wheat samples were cleaned using a Carter-Day dockage tester (Simon-Carter Company, Minneapolis, MN, USA). Hulled emmer wheat ‘97Emmer19’ and partially hulled line ‘2000EB4’ were de-hulled using an Agriculex SD-2 Spelt de-huller (Agriculex Inc., Guelph, Ontario, Canada) and later cleaned using the Carter-Day dockage tester, prior to further quality analysis.

Test weight (kg hL^{-1}) was determined on a plot basis using the AACC method 55-10 (AACC, 2000), using a Schopper chondrometer. Thousand-kernel weight was measured on cleaned grain sample as the weight (g) of 1000 seeds, following counting with an electronic seed counter (Agriculex Inc., Guelph, Ontario, Canada).

The Single Kernel Characterization System, SKCS Model 4100 (Perten Instruments North America, Springfield, IL, USA), was used to determine single kernel hardness index (SK-HI) (AACC method 55-31). The SKCS 4100 provides a rapid, objective measurement of the crushing force required to break kernels and was measured on 300 individual kernels (Sissons et al., 2000).

3.2.2 Grain Protein Concentration and SDS Sedimentation Volumes

Seed samples (60 g) from each plot were ground into whole wheat meal using a Udy Cyclone sample mill (Udy Corporation., Fort Collins, CO) fit with a 1-mm screen/mesh. Moisture content of ground meal were determined by the approved AACC method 44-15A (AACC, 2000). The protein concentration (%) of whole wheat meal was determined as 5.7 x total nitrogen on a LECO Model FP-528 combustion nitrogen analysis analyzer (LECO Instruments Corp., St Joseph, MI, USA) and reported on a 13.5% moisture basis (mb). For durum wheats, sodium dodecyl sulfate (SDS) sedimentation volumes (mL) were determined using a modification of AACC method 56-70 (AACC, 2000), using 3% SDS on whole wheat meal (14.0% mb). The use of the 3% SDS is not recommended for hexaploid wheat (Morris et al., 2007), hence 2% SDS solution was used for the bread wheat checks as recommended by Axford et al (1979).

3.2.3 Hagberg Falling Number and Yellow Pigment Concentration

The Hagberg Falling Number (Falling Number 1700 System, Perten Instruments, Sweden) was determined on whole wheat meal sample (14.0% mb) using the approved

AACC method 56-81B (AACC, 2000). Yellow pigment content (ppm or mg kg⁻¹) of whole wheat meal (14.0% mb) was assessed using a modification of AACC Method 14-50 (AACC, 2000). Instead of water-saturated n-butanol, 80% ethanol was used as a solvent. Yellow pigment concentration was calculated using absorbance values taken at 435 nm (Johnston et al., 1980) and was converted into mg kg⁻¹ using OD (Optical Density) x 20.79 (average extraction coefficient of lutein, zeaxanthin and β -carotene in 80% ethanol).

3.3 Quality Evaluation of Composite Samples

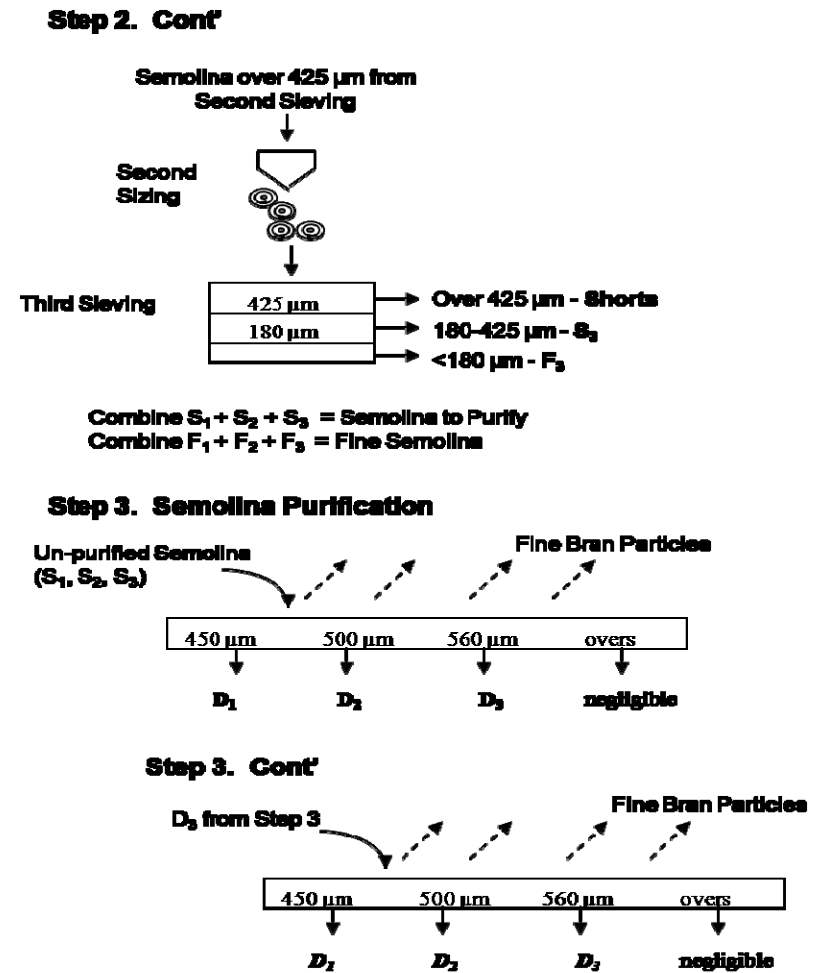
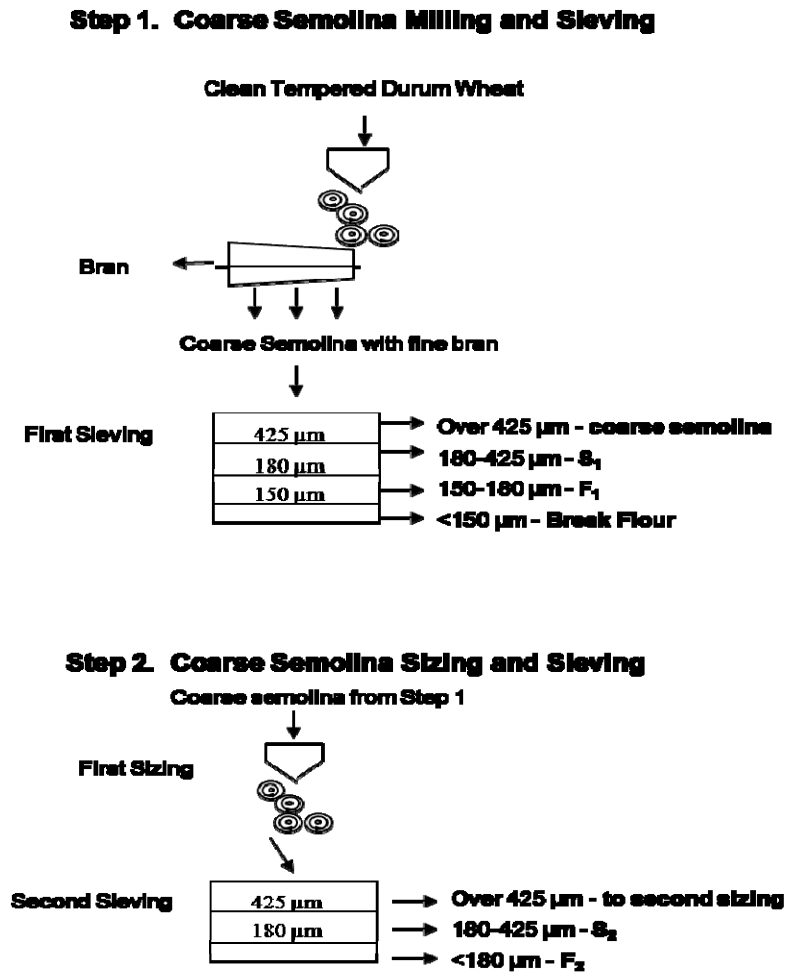
3.3.1 Wheat Milling

For each year of the study, a composite sample of each genotype was prepared by combining equal portions of grain from each of the nine replications (three replications from each environment). To provide sufficient sample for multiple quality tests, three millings were performed on 600 g samples. Semolina/flour from replicate millings of each composite sample was combined (blended) prior to quality testing.

3.3.1.1 Durum wheat experimental milling and purification

The durum wheat milling protocol used in this research was developed by Connie Briggs (Crop Development Centre, University of Saskatchewan) and is used routinely to determine semolina milling yield and to produce semolina for quality screening for the CDC's durum wheat breeding program. The seed moisture of the durum wheat samples was determined following AACC Method 44-15A (AACC, 2000), using approx. 5 g of seed ground using a Thomas-Wiley laboratory grinder (model 4, Arthur H. Thomas Co., Philadelphia, PA). Based on seed moisture, the durum wheat samples were tempered to 16% moisture for approx. 18 h prior to beginning the milling process. Durum wheat milling was done over three days (tempering, milling and purification on first, second and third days, respectively).

The durum wheat samples were milled into semolina using two Brabender Quadrumat Junior mills (C.W. Brabender Instruments, Inc., South Hackensack, NJ, USA) and purified using a CD2 semolina laboratory purifier (Chopin SA, Villeneuve-la-Garenne, France). A flow chart of the durum wheat milling procedure followed for this study is presented in Figure 1. The first Brabender Quadrumat Junior mill was equipped with semolina rolls (break cycle) to generate course semolina while the second Brabender



Step 4. Milling Yield Calculation

$$\text{Semolina Yield (as is)} = D_1 + D_2 + D_3 + D_4 + F_1 + F_2 + F_3$$

$$\text{Total Extraction (as is)} = \text{Break Flour Yld} + \text{Semolina Yld}$$

Determine moisture content of break flour and semolina and report milling results on a 14% moisture basis.

Figure 1. Flow chart of durum wheat milling and purification procedure followed for this study.

Quadrumat Junior mill was equipped with flour mill rolls to reduce particle size of the course semolina (Sizing cycle). Following the break cycle, the bran weight was recorded and discarded. The semolina between 180-425 μm from all the millings [S_1 (Step II), S_2 (Step IV), and S_3 (Step VI); Figure 1] was purified, as explained in Figure 1. The purified semolina was combined with the fine semolina [$< 180 \mu\text{m}$ from reduction milling i.e., F_1 (Step II), F_2 (Step IV), and F_3 (Step VI); Figure 1] to give final semolina product. The semolina yield combined with break flour yield was considered as the total extraction (%).

3.3.1.2 Bread wheat experimental milling

The three bread wheat samples were milled in triplicate (600 g each) on the same day. The seed moisture of the bread wheat samples was determined as per durum wheat (section 3.3.1.1). Based on seed moisture, bread wheat samples were tempered/conditioned to 14.5% moisture for approx. 18 h prior to milling. The samples were milled into flour using a Brabender Quadrumat Junior mill equipped with flour mill rolls according to AACC Method 26-21A (AACC, 2000). For the 2005 samples, the recovered bran from the mill was sifted for an extra 2 min using a Retsch Sieve Shaker fitted with a 250 μm sieve to recover any flour adhered to the bran. In 2006, a new Brabender Quadrumat Jr. flour mill was purchased and extra sifting of the bran was not necessary. Flour from replicate millings of each composite sample was combined (blended) prior to further testing.

3.3.2 Quality Tests Conducted on Semolina/Flour

Moisture content was measured on the flour/semolina (AACC method 44-15A; AACC, 2000) before conducting any of the quality tests and all data is reported on a 14.0% mb. Protein concentration of semolina/flour was estimated as 5.7 x total nitrogen as determined on a LECO Model FP-528 combustion nitrogen analysis analyzer (LECO Instruments Corp., St Joseph, MI, USA).

Bread wheat flour and durum wheat semolina colour was determined using an Agron reflectance color meter (M-45 Agron Process Analyzer, AGTRON INC, Reno, Nevada) according to AACC Method No. 14-30 (AACC, 2000). The colour of flour/semolina slurry from the Agron test was also assessed using the CIELab colour scale with a D65 illuminant on a HunterLab Miniscan spectrophotometer. After obtaining

the Agtron value, the dish was immediately placed on the colormeter opening and the L* a* b* measurements were taken. L* gives a measure of lightness/brightness (0 = black and 100 = white); a* measures greenness (-) to redness (+); and b* measures blueness (-) to yellowness (+). Each flour/semolina sample was evaluated twice for agtron and L* a* b* values.

The gluten index (GI) test was performed using AACC Method 38-12A (AACC, 2000). Repeated flooding of some semolina samples was experienced for the 1AS.1AL-1DL translocation lines. For those lines, the 2% sodium chloride (NaCl) solution was reduced from 4.8 mL to either 4.2 or 4.0 mL as recommended in the Glutomatic manual. Wet gluten content was determined following AACC Standard Method 38-12 (AACC, 2000) and dry gluten content was determined using AACC Standard Method 38-12A (AACC, 2000).

3.3.3 Rheological Properties of Semolina/Flour and Bake Test

3.3.3.1 Farinograph and Alveograph

Farinograph curves (C.W. Brabender Instruments, Inc., South Hackensack, NJ, USA) were generated according to AACC method 54-21 (AACC, 2000). The 50 g mixing bowl was used, in conjunction with the standard operating speed of 63 rpm. The curves were read manually and several parameters were recorded: farinograph water absorption (FAB, 14.0% mb), the amount of water required to centre the curve on the 500 BU line; stability (STA), the difference in time from when the top of the curve first reaches the 500 BU line (arrival time) to when it first leaves the 500 BU line (departure time); mixing tolerance index (MTI), the drop in the curve five minutes after peak development, measured in BU units; dough development time (DDT), the time required to reach peak dough development; and time to breakdown (TTB), the time from the start of mixing to the time at which the consistency decreases 30 BU from the peak.

Alveograph curves were obtained following AACC Method 54-30A (AACC, 2000) using a Chopin Alveograph (Model MA82, Chopin SA, Villeneuve-la-Garenne, France). Average values for five dough pieces per composite sample were obtained for overpressure or resistance to extension (P), abscissas at rupture or extensibility (L), configuration ratios (P/L) and deformation energy (W).

3.3.3.2 Canadian Short Process (CSP) bake test

The CSP bake method (a short fermentation method) (Preston et al., 1982) was used for this study. The formulation (14.0% mb) contained 100 g semolina/flour, 2.4 g salt, 4.0 g sugar, 3.0 g shortening (Crisco all-vegetable shortening), 0.6 g malt powder (Dover Industries limited, Saskatoon), 4.0 g whey powder (Dover Industries Limited, Saskatoon), 150 ppm ascorbic acid (BDH Merck Analar grade) as oxidant, fresh compressed yeast (Fleishman compressed yeast), and optimum water (based on farinograph water absorption).

In both 2005 and 2006 baking trials, semolina/flour was baked in three replications (triplicate) on different days. For 2005 baking trials, each replication was baked over two days, whereas for 2006 baking trials, each replication (all samples) was baked on the same day. Ingredients were mixed to slightly past peak in a GRL 200 Mixer (Muzeen and Blythe Ltd. Winnipeg, Manitoba) at 165 rpm, and peak mixing time (PMT, min) and the mixing energy (Wh/kg) were recorded. After mixing, the dough was rounded by hand and placed in fermentation cabinet controlled at 34°C and 85% relative humidity. The dough was punched by hand at 15 min, allowed to proof a further 15 min and panned at 30 min. Panned dough was proofed for 70 min and baked at 400°C for 25 min. After baking, the loaves were cooled at room temperature for 30 min and loaf volumes were measured by rapeseed displacement using a National Loaf Volumeter (National Manufacturing Company, Lincoln, NB, USA) (Cathcart and Cole, 1938). Loaves were scored (0-5 scale) for general loaf shape and appearance i.e., external loaf quality (Table 2). The loaves were then sliced and visually assessed (0-5 scale) for internal loaf quality i.e., crumb colour and crumb structure (Table 2).

3.4 Electrophoresis Procedures

3.4.1 Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)

For all cultivars and breeding lines, allelic composition at glutenin loci (HMW-GS and LMW-GS) were determined by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using 10% separating gel / 4% stacking gel and 12% separating gel / 4% stacking gel (Singh et al. 1991). For 12% separating gels / 4% stacking gels, bread wheat cultivar ‘Neepawa’ and Bio-Rad protein size standards (HMW and LMW-size standards) were used as standards to identify glutenin subunits.

Table 2. Guidelines for scoring the Experimental Loaves

Score	External loaf quality		Internal loaf quality	
	Loaf Shape and Appearance		Crumb Colour	Crumb Structure
0	No rise, the loaf resembles a square brick		Bright yellow	Very coarse, thick cell walls, open structure, may have large holes, and with uneven, irregular cell size
1	Very little oven rise, may have uneven or irregular shape, bumpy top, coarse side walls, no break and shred ^a		Very yellow	Very coarse, thick cell walls and open structure, cell size distribution appears more regular
2	Small or dwarf appearance, irregular shape, possibly flat topped, minimal or rough break and shred		Slightly yellow	Coarse but with somewhat even cell size distribution, or very open with large, uneven cell size or very fine textured with small, round cells
3	Normal appearance with rounded top and some break and shred		Creamy	Even textured, may be slightly open with fairly thin cell wall, cell size distribution somewhat uneven
4	Very good loaf with rounded top, high break and shred, smooth side walls		White, maybe slightly creamy	Fine and even, thin cell walls, slightly open in centre, cells around the outside are elongated and fine
5	Large pup loaf with very high break and shred, yet retaining a straight, upright appearance		Bright white	Cell walls are very thin, elongated around the outside, with cell size distribution very even, no larger cells noticeable in the centre

^aBreak and shred = The portion of the loaf between the top and the sides that shreds during baking.

Twelve percent separating gels / 4% stacking gels were used for all the genotypes except for the ‘Chinook’, ‘Marquis’, ‘97Emmer19’, and emmer-derived breeding lines. For these lines 10% separating gels / 4% stacking gels were used, since the separation of *Bx14* and *By15* (at *Glu-B1* locus) glutenin subunits of some emmer and emmer breeding lines was not distinct (difficult to characterize) with 12% separating gels / 4% stacking gels. For 10% separating gels / 4% stacking gels, bread wheat cultivars ‘Chinook’ and ‘Marquis’ were used as standards to identify glutenin subunits.

3.4.1.1 Glutenin protein extraction

Two or three seeds were ground by hand using a mortar and pestle and for each sample, a separate cleaned mortar and pestle were used to avoid cross-contamination. One mL of freshly made extraction buffer solution [prepared by mixing 24 mL water, 10.2 mL extracting buffer stock solution (prepared by mixing 20 mL glycerol, 12.5 mL stacking-gel buffer solution i.e., 1.0 M Tris with pH 6.8 adjusted with Hydrochloric acid by a pH meter, 24.1 mL water, 4 g SDS, and 20 mg bromophenol blue), and 1.8 mL β -mercaptoethanol] was added to 40 mg of ground sample. The mixture was allowed to stand at room temperature for 2 h with occasional shaking on a vortex mixer. The mixture was then heated for 2.5 min in a boiling water bath, then centrifuged for 15 min at 13,000 rpm, and was allowed to cool to room temperature. The mixture was allowed to settle and an aliquot of clear supernatant was used as the experimental protein extract.

3.4.1.2 SDS-PAGE gel preparation and electrophoresis run

A vertical slab electrophoresis instrument (PROTEAN II xi Cell, Bio-Rad Laboratories, Hercules, CA, USA) which accommodates gels that are 140 mm wide x 160 mm high x 1.5 mm thick was used. Each gel accommodates 15 samples and a 15 well comb of 1.5 mm thickness was used. The glass plates were washed with de-ionized water thoroughly, were coated with 1:200 Kodak photofluor, and allowed to dry. The glass plates were positioned in the gel-forming cassette. The 4% stacking gel [3.3 mL of 30% acrylamide / bisacrylamide solution, 250 μ l of 10% SDS, 6.3 mL of 0.5 M Tris-HCl at pH 6.8, 15 mL distilled deionized water, 125 μ l of 10% APS (Ammonium persulphate), and 25 μ l of TEMED (N,N,N',N'- tetramethyl ethylene diamine) was mixed to prepare 25 mL of 4% stacking gel solution], 12% separating gel [40 mL of 30% acrylamide/bisacrylamide solution, 25 mL of 1.5 M Tris-Hcl at pH 7.8, 1 mL of 10%

SDS, 33.5 mL distilled deionized water, 500 µl of 10% Ammonium persulphate, and 50 µl of TEMED (N,N,N,N-tetramethyl ethylene diamine) was mixed to prepare 100 mL of 12% separating gel solution], and/or 10% separating gel [33 mL of 30% acrylamide/bisacrylamide solution, 25 mL of 1.5 M Tris-HCl at pH 7.8, 1 mL of 10% SDS, 40.5 mL distilled deionized water, 500 µl of 10% Ammonium persulphate, and 50 µl of TEMED (N,N,N,N-tetramethyl ethylene diamine) was mixed to prepare 100 mL of 10% separating gel solution] were prepared as explained above.

Ten µl of protein extract was loaded and electrophoresis carried out at 20°C temperature (regulated by water bath at $20 \pm 1^\circ\text{C}$) at a constant current of 20 mA for 21 h, per gel.

3.4.1.3 Glutenin protein detection by Silver Staining

Following electrophoresis, the gels were removed from the glass plates, placed into a gel staining container and immersed in de-ionized water. Proteins were fixed by adding 300 mL of fixing solution (prepared by mixing 150 mL of methanol, 30 mL of glacial acetic acid, and 120 mL of de-ionized water) and gently shaken for 30 min at 24°C. After discarding the fixing solution, the gels were incubated (for 30 min at 24°C temperature with gentle shaking) with 300 mL of the second fixing solution, prepared by mixing 16.5 mL of methanol, 22.5 mL of glacial acetic acid, and 261 mL of de-ionized water. The gels were then rinsed twice with 300 mL of de-ionized water for 10 min. After discarding the water, 300 mL of freshly prepared 0.0005% DTT (Dithiothreitol) solution was used to incubate the gels for 30 min with gentle shaking. The DTT solution was discarded and 300 mL of 0.1% AgNO₃ (Silver nitrate) was used to stain the gels for 30 min with gentle shaking. The gels were rinsed for 20 sec with de-ionized water. 150 mL of developing solution (prepared by using 13.2 g sodium carbonate and 0.061 mL of formaldehyde to 300 mL of de-ionized water) was added and gently hand agitated for 30 sec. The gels were rinsed for a second time with 150 mL of developing solution and 150 mL of developing solution was again added for final band development. Once the bands reached the desired intensity (after 15-30 min), 5 mL of 72% citric acid solution was added to terminate the reaction. The glutenin protein subunits were scored based on their molecular weights, according to Payne and Lawrence (1983) and Ng et al. (1988b).

3.4.2 Acid - Polyacrylamide Gel Electrophoresis (A-PAGE)

For all cultivars and breeding lines, allelic compositions at gliadin loci (α , β , γ , and ω -gliadins) were fractionated by acid (pH 3.1) polyacrylamide gel electrophoresis (A-PAGE) (Bushuk and Zillman, 1978).

3.4.2.1 Gliadin protein extraction

Two or three seeds were ground by hand using a mortar and pestle and for each sample, a separate cleaned mortar and pestle were used to avoid cross-contamination. 500 μ l of 70% ethanol (prepared by mixing 36.85 mL of 95% ethanol and de-ionized water was added to make the final volume to 50 mL) was added to 100 mg of ground sample and mixed for 3 h. The mixture was centrifuged at 13,000 rpm for 15 min and 200 μ l of GMG (Methylene Green) solution (prepared by dissolving 10 mg methylene green in 8 mL aluminum lactate buffer and by adding 6 mL glycerol) was added to the 200 μ l of the diluents. The mixture (gliadin protein extract) was stirred and stored at 4°C.

3.4.2.2 Acid-PAGE gel preparation and electrophoresis run

A vertical slab electrophoresis instrument (PROTEAN II xi Cell, Bio-Rad Laboratories, Hercules, CA, USA) was used for Acid-PAGE. Prior to pouring the gel, the glass plates were washed with 1% SDS solution and rinsed with de-ionized water thoroughly. Later, the glass plates were coated with 1:200 Kodak photofluor and allowed to dry. The gel solution was prepared by weighing 20 g acrylamide, 750 mg bis-acrylamide, and 250 mg ascorbic acid dissolved in 50 mL of Aluminium lactate buffer (prepared by dissolving 20 g aluminum lactate in 1 L of de-ionized water, later adjusting the pH to 3.1 with lactic acid and finally making up the volume to 8 L). To this solution 1 mL of FeSO₄ (Ferrous sulphate) solution (prepared by dissolving 20 mg FeSO₄ in 1 mL aluminum lactate buffer and volume made to 5 mL with aluminum lactate buffer) was added. The volume was made to 250 mL in the volumetric flask with de-ionized water. The gel solution was stored in glass bottle was stable up to one week at 4°C. To prepare the gel, 500 μ l of 1% H₂O₂ (Hydrogen peroxide) solution was added to 50 mL of gel solution, while stirring for 5 sec. The gels were poured immediately between the glass plates and a 15 well comb was inserted.

Ten μ l of protein extract was loaded and electrophoresis carried out at 20°C (regulated by water bath at 20 \pm 1°C) at a constant current of 25 mA for 4 h, per gel.

3.4.2.3 Gliadin protein detection by Coomassie Brilliant Blue (CBB-R 250) solution

The gels were removed from the glass plates by placing the gels in a gel staining container, immersing in de-ionized water. The gels were washed with 12% TCA (Trichloroacetic acid) for 5 min with gentle shaking. After discarding the TCA solution, the gels were washed with de-ionized water and 240 mL of CBB-R 250 staining solution (prepared by dissolving 400 mg CBB-R 250 in 100 mL 95% ethanol and later pipetting 10 mL of Coomassie brilliant blue solution to 250 mL 10% trichloroacetic acid solution) was poured over the gels and allowed to stand overnight with gentle shaking. After 16 h, the CBB-R 250 staining solution was discarded and the gels were washed twice with de-ionized water. The gliadin protein subunits were scored based on their relative mobility (RM) values (Xu et al., 2005).

Durum wheat genotype 'Stewart-63' and 1AS.1AL-1DL translocation line 'L252' were used as reference cultivars to calculate the relative mobility of the gliadin band(s) (Ng et al., 1988b). Both the reference samples contain the γ -42 gliadin band (Ng et al., 1988b; Klindworth et al., 2005).

3.5 Statistical Analysis

3.5.1 Non-composite Samples

For each location and year, statistical analyses were conducted using SAS Institute Inc. Software, version 9.1 (SAS Institute, 2001). Replication was considered as random and genotype as fixed effect. Least square (LS) means and Least Significant Difference (LSD at 5 % significance level) were generated using the SAS PROC MIXED procedure.

Combined data from both years were subjected to analysis of variance using the SAS PROC MIXED procedure with year, location, year*location, replication(year*location), genotype*location, genotype*year, and genotype*location*year as random effects and genotypes as fixed effect. LSD (P = 0.05) was reported for genotype LS means comparison.

3.5.2 Composite Samples

The average and standard deviation (of triplicate measures for milling yield, loaf volume, loaf shape, crumb colour, crumb structure, and peak mixing time and duplicate measures for Agtron, L*, a*, b*, gluten index, wet gluten content, dry gluten content, and flour/semolina protein concentration) of quality tests conducted on flour/semolina, for individual year's composite samples, were calculated using Excel (Microsoft

Corporation, Microsoft Office Excel 2007). The combined composite data from 2005 and 2006 were subjected to ANOVA (SAS PROC MIXED procedure) with year and genotype*year as random effects and genotypes as fixed effect. LSD (P = 0.05) was reported for genotype LS means comparison.

The average and standard deviation for alveograph curves (for five dough pieces per composite sample) were calculated in Excel for P , L , W and P/L . The farinograph (FAB, DDT, MTI, TTB and STA) combined composite data (2005 and 2006) were subjected to ANOVA (SAS PROC MIXED procedure) with year as random effect and genotypes as fixed effect. LSD (P = 0.05) was reported for genotype LS means comparison. The alveograph (P , L , P/L and W) combined composite data (2005 and 2006) were subjected to ANOVA (SAS PROC MIXED procedure) with year and year*genotype as random effects and genotypes as fixed effect. LSD (P = 0.05) was reported for genotype LS means comparison.

3.5.3 Correlation Coefficients among Inter-related Quality Traits

Genotypic LS means were used to estimate the Pearson correlation coefficients (SAS Proc Corr statement) among inter-related quality traits, once with data only from tetraploid wheats (durum wheats, '97Emmer19', emmer-derived breeding lines, and 1AS.1AL-1DL translocation lines) and once by excluding the USDA-ARS 1AS.1AL-1DL translocation lines.

3.5.4 Contrast analyses between Protein Subunits and Loaf Volume

The genotypic classes with different protein subunits and their corresponding loaf volumes were compared using contrast (single degree of freedom) statement in SAS PROC MIXED procedure. The difference in loaf volume estimates and standard error of the difference (SED) were generated for LV of genotypic classes with different protein subunits.

4. Results

4.1 Quality Evaluation of Non-composite Samples (Whole Grain Quality Measures)

4.1.1. Test Weight (TWT)

For individual environments across years, the replication effect was non-significant and the genotypic effect was statistically significant for TWT (Appendix A1). Averaged over all environments and years (combined ANOVA), genotypes differed in TWT. The variance estimates for the two-way interactions between location and year, and location and genotype, were not statistically different from zero ($P < 0.05$). However, the genotype x year and genotype x year x location interaction variance estimates were significantly greater than zero ($P < 0.05$), although the three-way interaction estimate was small (Appendix A1).

All of the genotypes showed higher TWT in the 2006 growing season compared to the 2005 growing season (Table 3). ‘97Emmer19’ showed significantly lower TWT than bread wheat and durum wheat varieties in all testing environments (Table 3). In contrast, the majority of emmer-derived breeding lines had similar TWT to the durum and bread wheat varieties evaluated (Table 3). In most environments, ‘X.98.142.17’ showed significantly lower TWT than the durum wheat checks ‘Commander’, ‘AC Navigator’ and ‘Strongfield’, and ‘AC Barrie’, the bread wheat variety with the highest TWT (Table 3). The CIMMYT 1AS.1AL-1DL translocation lines, grown only during the 2005 growing season, showed no statistical differences in TWT in the individual environments, except at GDL (Table 3). The USDA-ARS 1AS.1AL-1DL translocation lines showed no significant difference in TWT in the individual environments except at GDL during the 2005 growing season (Table 3). However, averaged over all environments, no significant differences in TWT were observed (Table 3).

The durum wheat check cultivars ‘Strongfield’, ‘Commander’, and ‘AC Navigator’ all had high TWT, similar to the bread wheat checks ‘AC Barrie’, ‘CDC Teal’ and ‘AC Superb’, regardless of environment (Table 3). ‘Stewart-63’ consistently displayed greater TWT than all bread wheat checks, although ‘AC Superb’ at SF (2005) and ‘AC Barrie’ at GDL (2005) and SF (2005) showed statistically similar TWT to ‘Stewart-63’. ‘Arcola’ had poor TWT compared to other durum genotypes except ‘WB881’ and ‘Commander’ (Table 3).

Table 3. Least square means for Test Weight (kg hL⁻¹), 1000 Kernel Weight (g), and Grain Hardness Index of bread wheat checks (AC Barrie, CDC Teal, and AC Superb) and tetraploid wheats (durum, emmer, 1AS.1AL-1AD translocation, and emmer-derived breeding lines) planted during the 2005 and 2006 growing seasons at Goodale (GDL), Kernen (KER) and Seed Farm (SF) locations.

Genotype	Test weight (kg hL ⁻¹)							1000-Kernel weight (g)							Grain Hardness Index						
	GDL		KER		SF		C ^d	GDL		KER		SF		C	GDL		KER		SF		C
	'05 ^a	'06 ^b	'05	'06	'05	'06		'05	'06	'05	'06	'05	'06		'05	'06	'05	'06	'05	'06	
BREAD WHEAT CHECK CULTIVARS																					
AC Barrie	79.9	81.2	78.1	81.5	78.5	81.7	80.2	39.0	37.9	37.1	37.9	33.9	37.1	37.2	53	50	55	47	54	53	52
CDC Teal	79.3	79.8	77.1	81.1	77.5	80.5	79.2	38.3	37.1	36.0	36.2	37.7	36.0	36.9	51	51	52	49	49	56	51
AC Superb	79.5	81.1	76.8	81.6	78.6	80.9	79.8	43.5	43.0	38.3	40.9	38.3	40.6	40.8	55	45	56	48	58	49	52
1AS.1AL-1DL TRANSLOCATION LINES																					
04EDUYT-42	79.2	-	76.9	-	78.7	-	-	41.1	-	37.4	-	39.8	-	-	80	-	77	-	77	-	-
04EDUYT-43	80.7	-	77.1	-	77.9	-	-	42.2	-	39.7	-	39.3	-	-	80	-	78	-	82	-	-
04IDSN-107	79.8	-	77.3	-	78.3	-	-	39.1	-	40.5	-	39.1	-	-	82	-	78	-	78	-	-
04IDSN-111	79.2	-	76.6	-	77.6	-	-	39.6	-	38.8	-	39.1	-	-	81	-	78	-	83	-	-
L092	78.9	80.8	78.1	81.2	79.2	80.4	79.1	39.4	42.1	36.0	43.1	40.5	44.2	40.9	78	64	82	68	72	71	73
L252	77.8	79.9	78.0	80.6	77.7	80.7	79.8	42.2	42.9	38.6	41.8	42.3	44.3	42.0	76	60	80	68	75	69	71
S99B33	78.7	80.5	77.8	80.7	78.6	-	-	40.8	41.1	36.9	42.2	39.3	-	-	77	67	84	72	75	-	-
S99B34	78.9	80.1	77.8	81.4	79.4	80.5	79.7	40.0	41.0	36.1	41.1	40.3	40.6	39.9	78	67	82	71	74	65	73
DURUM WHEAT CULTIVARS																					
Strongfield	80.4	81.7	79.1	82.3	79.7	82.4	80.9	47.9	44.6	43.7	48.4	46.6	48.0	46.5	71	69	74	63	72	67	69
WB881	78.6	80.2	75.1	80.7	77.9	80.7	78.9	47.5	45.2	42.1	48.5	44.0	48.2	45.9	72	69	76	63	72	68	70
Commander	79.3	80.7	77.3	82.0	79.5	81.7	80.1	49.2	47.8	46.2	51.4	48.8	50.4	49.0	74	69	76	66	74	68	71
AC Navigator	80.4	81.3	78.3	82.6	79.6	82.3	80.8	48.9	50.6	46.4	49.7	48.0	50.0	48.9	72	68	74	63	72	68	70
DT724	79.9	82.3	78.8	82.0	80.1	82.1	80.9	46.7	45.2	43.4	45.3	44.3	48.2	45.5	75	72	78	70	76	69	73
Stewart-63	80.7	84.9	80.0	84.3	80.6	82.9	82.2	51.7	51.8	46.8	50.4	44.9	52.4	49.7	74	71	74	69	74	65	71
Arcola	77.5	79.3	75.5	80.3	77.5	78.8	78.1	44.3	46.1	42.3	48.1	47.0	47.1	45.8	71	62	73	62	66	66	67
'97EMMER19' AND EMMER-DERIVED BREEDING LINES																					
97Emmer19	71.6	75.4	71.1	78.6	68.6	77.5	73.8	30.7	25.6	32.1	35.5	29.4	33.3	31.1	70	53	71	52	66	59	62
2000EB4	77.1	79.4	76.7	80.8	77.6	81.5	78.9	37.0	40.7	36.0	44.1	35.2	45.1	39.7	72	61	75	59	72	62	67
X.98.142.17	76.3	79.6	72.1	80.8	73.2	79.5	76.9	41.7	44.9	38.8	50.3	40.1	46.4	43.7	76	69	76	63	74	69	71
X.98.142.18	74.6	-	72.4	-	74.6	-	-	39.6	-	39.4	-	39.4	-	-	72	-	75	-	72	-	-
P.01.64.31	79.9	81.6	76.2	81.7	80.3	81.5	80.2	48.8	46.0	44.4	48.5	47.2	48.2	47.2	72	69	75	64	71	68	70
P.01.64.39	79.4	81.4	77.5	82.8	78.3	82.5	80.3	44.9	43.8	40.1	46.8	44.3	46.8	44.4	74	71	79	68	74	72	73
P.01.64.62	77.1	80.1	74.8	81.5	75.6	81.0	78.3	43.0	40.6	39.0	43.2	42.1	41.7	41.6	77	69	80	69	75	74	74
05Emmereg-01	-	79.1	-	79.7	-	79.5	-	-	41.8	-	41.9	-	42.7	-	-	65	-	63	-	69	-
05Emmereg-03	-	79.0	-	79.3	-	78.7	-	-	43.5	-	48.4	-	44.0	-	-	71	-	64	-	72	-
05Emmereg-10	-	79.6	-	80.3	-	79.9	-	-	44.4	-	44.9	-	44.7	-	-	72	-	67	-	70	-
05Emmereg-26	-	77.6	-	79.5	-	78.5	-	-	49.1	-	54.0	-	50.5	-	-	61	-	56	-	63	-
LSD _{0.05} ^c	1.0	1.2	1.2	1.0	2.2	1.1	1.9	2.8	2.2	2.2	2.8	3.2	4.0	3.3	3	5	2	2	5	6	5

^a'05 = 2005 growing season ^b'06 = 2006 growing season ^cLSD = Least significant difference

^dC = Combined data across environments (GDL, KER, and SF) and/or growing seasons (2005 and 2006).

4.1.2 Thousand Kernel Weight (KWT)

For individual environments across years, the replication effect was non-significant and the genotype effect was statistically significant for KWT (Appendix A1). For the combined ANOVA, the variance estimates for the two-way interactions between location and year, and location and genotype were not statistically different from zero ($P < 0.05$). The genotype x year x location interaction variance estimates were significantly greater than zero ($P < 0.05$), although the three-way interaction estimate was small (Appendix A1).

Averaged over environments, the KWT ranged from 31.1 g for '97Emmer19' to 49.7 for 'Stewart-63' (Table 3). '97Emmer19' consistently showed significantly lower KWT than the durum and bread wheat checks ($P < 0.05$), except at KER (2006) and SF (2006) (Table 3). At these environments, '97Emmer19' showed no significant difference for KWT from 'AC Barrie' and 'CDC Teal' bread wheat checks (Table 3). All of the emmer-derived breeding lines displayed improved test weight over '97Emmer19', but over two years of testing, only 'P.01.64.31' showed KWT similar to the durum wheat checks (Table 3). '05Emmereg-26', evaluated only in 2006, showed significantly higher KWT than other emmer-derived breeding lines and bread wheat checks ($P < 0.05$) (Table 3). The CIMMYT and 1AS.1AL-1DL translocation lines showed reduced KWT compared to durum wheat checks 'Strongfield', 'Commander', and 'AC Navigator' (Table 3). As expected, the durum wheat checks had significantly higher KWT than the bread wheat varieties ($P < 0.05$) (Table 3). At GDL (2006), 'Strongfield' (44.6 g) showed significantly lower KWT than most durum genotypes ($P < 0.05$), but no significant difference was seen between 'Commander' (47.8 g) and 'Arcola' (46.1 g) (Table 3).

4.1.3 Grain Hardness (HI)

The combined ANOVA across all environments revealed significant differences among genotypes (Appendix A2). Averaged over all environments, '97Emmer19' showed a significantly softer kernel than the durum checks ($P < 0.05$), and was similar to 'Arcola' and '2000EB4' in kernel texture at the GDL (2005) and SF (2006) environments. In individual environments, the emmer-derived breeding lines had significantly higher HI than '97Emmer19' ($P < 0.05$) (Table 3). Averaged over all environments, 'P.01.64.62' showed significant higher HI than most emmer-derived

breeding lines ($P < 0.05$) (Table 3). USDA-ARS 1AS.1AL-1DL translocation line ‘L252’ showed significantly lower HI than other USDA-ARS 1AS.1AL-1DL translocation lines ($P < 0.05$) only in the GDL (2006) and KER (both the growing seasons) environments (Table 3).

Averaged over all environments, the HI values for bread wheat ranged from 51 to 52, with durum checks having HI values of 69 or greater (Table 3). Among the durum wheat genotypes, ‘Arcola’ showed significantly softer ($P < 0.05$) grain than most durum genotypes (Table 3). As expected, the bread wheat checks showed softer endosperm, owing to the presence of the puroindoline a (*Pina-D1*) and puroindoline b (*Pinb-D1*) genes on the short arm of chromosome 5D (Gautier et al., 2000; Tranquilli et al., 2002).

4.1.4 Grain Protein Concentration (GPC)

The combined ANOVA across environments revealed a significant genotype x environment interaction for GPC as the genotype x year x location interaction variance estimate was significantly greater than zero (Appendix A2). Averaged over all genotypes, GPC was highest at the KER and SF environments in 2005 (Table 4). In all environments, significant genotypic effects ($P < 0.01$) were detected for GPC (Appendix A2). Data for GPC at the SF in both years was highly variable, with LSD (0.05) estimates of 1.3 and 2.1% for 2005 and 2006, respectively (Table 3). Highly variable GPC data was also seen in the GDL environment in the 2006 growing season with an LSD estimate of 1.7%. Data was tested for outliers using a standardized Student’s t-test and no outliers were detected for any of the locations (Data not shown). The largest range in GPC was at SF in 2005 with LS means ranging from 10.7% - 16.1%.

Averaged over environments, ‘97Emmer19’ had high GPC, similar to ‘CDC Teal’ and ‘AC Barrie’, but not significantly different ($P < 0.05$) from ‘Strongfield’ (Table 4). Averaged over environments, ‘97Emmer19’ (14.6%) and ‘P.01.64.39’ (14.6%) showed statistically significant higher GPC than other emmer-derived breeding lines ($P < 0.05$) (Table 4). In most environments, ‘2000EB4’ had numerically lower GPC than ‘97Emmer19’, but the differences were not significant. ‘05Emmereg-03’ showed significantly higher GPC than other emmer-derived breeding lines at the GDL (13.8%), KER (16.5%), and SF (15.8%) locations in 2006 ($P < 0.05$) (Table 4). In most environments, the USDA-ARS 1AS.1AL-1DL translocation lines ‘L092’ and ‘L252’, the

Table 4. Least square means for Grain Protein Concentration (13.5% mb) and SDS Sedimentation volumes (mL) of bread wheat checks (AC Barrie, CDC Teal, and AC Superb) and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines) planted during the 2005 and 2006 growing seasons at Goodale (GDL), Kernen (KER) and Seed Farm (SF) locations.

Genotype	Grain Protein concentration (%)							SDS Sedimentation volumes (mL)						
	GDL		KER		SF			GDL		KER		SF		
	'05 ^a	'06 ^b	'05	'06	'05	'06	C ^d	'05	'06	'05	'06	'05	'06	C
BREAD WHEAT CHECK CULTIVARS														
AC Barrie	14.8	13.3	15.2	15.4	13.8	15.9	14.7	73	59	86	73	69	64	71
CDC Teal	15.0	12.8	15.6	15.3	14.2	14.6	14.6	86	68	90	77	81	68	78
AC Superb	14.2	13.7	14.7	15.8	13.8	13.3	14.2	73	61	83	69	72	57	69
1AS.1AL-1DL TRANSLOCATION LINES														
04EDUYT-42	11.2	-	11.7	-	10.9	-	-	18	-	17	-	17	-	-
04EDUYT-43	11.1	-	11.8	-	11.5	-	-	19	-	17	-	18	-	-
04IDSN-107	10.8	-	11.7	-	10.7	-	-	18	-	17	-	17	-	-
04IDSN-111	11.3	-	11.7	-	11.4	-	-	19	-	17	-	18	-	-
L092	14.8	12.1	14.5	14.1	13.6	15.0	14.0	53	47	57	53	55	48	52
L252	15.7	11.8	14.6	14.9	16.1	15.2	14.7	42	37	44	43	44	39	41
S99B33	15.1	12.6	14.8	14.8	14.8	-	-	53	46	52	50	57	-	-
S99B34	15.7	12.9	14.4	14.7	14.5	13.0	14.2	56	49	53	52	53	42	51
DURUM WHEAT CULTIVARS														
Strongfield	15.2	13.3	14.5	14.4	14.9	13.3	14.3	42	41	41	46	42	41	42
WB881	12.9	11.3	14.5	13.7	13.4	13.5	13.2	54	40	50	51	54	47	49
Commander	13.4	11.0	13.3	14.4	12.4	13.6	13.0	49	42	44	55	46	47	47
AC Navigator	13.2	12.4	13.4	14.3	12.8	14.3	13.4	40	36	36	47	36	39	39
DT724	14.3	12.5	13.8	14.5	13.5	13.7	13.7	40	40	44	45	38	39	41
Stewart-63	13.9	11.1	13.8	13.6	13.5	13.6	13.2	20	18	20	21	17	18	19
Arcola	14.3	11.9	14.9	13.6	13.5	14.0	13.7	43	32	32	38	38	41	37
'97EMMER19' AND EMMER-DERIVED BREEDING LINES														
97Emmer19	14.7	13.7	15.6	14.1	14.2	15.2	14.6	57	42	39	50	48	50	47
2000EB4	14.5	13.4	15.5	14.2	14.0	14.0	14.3	67	58	62	67	68	57	63
X.98.142.17	12.7	11.9	13.2	14.5	13.2	13.2	13.1	60	54	62	64	53	52	58
X.98.142.18	12.3	-	13.4	-	13.1	-	-	51	-	50	-	59	-	-
P.01.64.31	13.1	11.4	13.9	13.8	13.2	13.1	13.0	55	39	49	53	53	49	50
P.01.64.39	14.3	13.1	14.8	14.6	15.1	15.5	14.6	48	43	46	51	49	45	47
P.01.64.62	13.6	11.7	13.6	12.7	12.9	13.9	13.0	52	44	51	51	50	48	49
05Emmereg-01	-	10.3	-	12.9	-	12.9	-	-	33	-	54	-	50	-
05Emmereg-03	-	13.8	-	16.5	-	15.8	-	-	35	-	42	-	40	-
05Emmereg-10	-	12.3	-	14.1	-	14.0	-	-	46	-	58	-	53	-
05Emmereg-26	-	12.3	-	14.0	-	13.5	-	-	43	-	51	-	49	-
LSD ^c _{0.05}	0.6	1.7	0.6	0.6	1.3	2.1	0.8	5	6	4	3	5	7	8

^a'05 = 2005 growing season

^b'06 = 2006 growing season

^cLSD = Least significant difference

^dC = Combined data across environments (GDL, KER, and SF) and/or growing seasons (2005 and 2006)

GPC were not significantly different from ‘Strongfield’ (Table 4).

Averaged over environments, ‘Strongfield’ showed significantly higher GPC than ‘Commander’, ‘AC Navigator’ and ‘WB881’ ($P < 0.05$), and displayed similar GPC to ‘AC Superb’ (Table 4). At the GDL location in the 2005, ‘WB881’ (12.9%) had significantly lower GPC than other durum genotypes ($P < 0.05$) although no significant difference was detected between ‘WB881’, ‘Commander’ and ‘AC Navigator’ when averaged over all environments (Table 4).

4.1.5 SDS Sedimentation Volumes

For individual environments, the replication effect was non-significant and the genotype effect was statistically significant for SDS sedimentation volume (Appendix A3). For the combined analyses of variance across environments, genotypes differed in SDS sedimentation volume (Appendix A3). The variance estimates for the genotype x year ($P < 0.05$) and genotype x year x location ($P < 0.01$) interaction variance estimates were greater than zero, indicating a significant genotype x environment interaction. However, the correlation coefficients between LS means of SDS sedimentation volumes ranged from 0.89 ($P < 0.001$) to 0.98 ($P < 0.001$), indicating relative differences between genotypes were consistent from environment to environment.

‘2000EB4’ is derived from a cross between ‘WB881’ and ‘97Emmer19’ (Table 1). In most environments, SDS sedimentation volumes of the two parental lines were similar, despite varying GPC (Table 4). However, ‘2000EB4’ showed significantly higher SDS sedimentation volumes ($P < 0.05$) than both of its parents in all environments (Table 4). ‘2000EB4’ was selected for high SDS sedimentation volume during the development of that line (P. Hucl, personal communication), which may indicate that transgressive segregation was present in the segregating breeding population. The presence of transgressive segregation would imply that both ‘WB881’ and ‘97Emmer19’ are contributing alleles to elevated SDS sedimentation. Alternatively, ‘2000EB4’ has significantly higher GPC than ‘WB881’, which may also explain the higher SDS sedimentations observed in ‘2000EB4’ as SDS sedimentation values are influenced by GPC (Fowler and De La Roche, 1975; Autran and Galterio, 1989; Galterio et al., 1993; Novaro et al., 1997; Porceddu et al., 1998). However, ‘X98.142.17’ had significantly lower GPC ($P < 0.05$) than ‘2000EB4’, and similar SDS sedimentation volumes (Table 4).

‘X98.142.17’ was selected from the cross ‘WB881’ *2/ ‘97Emmer19’, supporting the hypothesis that ‘97Emmer19’ does contain some alleles that when recombined with those from ‘WB881’, result in elevated SDS sedimentation.

Among the durum wheat cultivars, large variation in SDS sedimentation values was evident. ‘Stewart-63’ and the CIMMYT translocation lines had very weak gluten, with SDS values below 21 mL in all environments. ‘Stewart-63’ possesses the LMW-1 glutenin subunit, which is known to have a negative effect on gluten strength in durum wheat (see Literature Review section 2.2.2.2). Interestingly, USDA-ARS 1AS.1AL-1DL translocation line ‘L252’ also carries the LMW-1 type glutenin subunit, but the SDS sedimentation volumes of ‘L252’ were significantly higher than those of ‘Stewart-63’, and similar to the remaining 1AS.1AL-1DL translocation lines which possess the LMW-2 banding pattern (Table 4). ‘L252’ carries the 1AS.1AL-1DL translocation which is known to improve gluten strength in hexaploid wheat (see Literature Review section 2.2.2.2), which appears to compensate for the negative effect of the LMW-1 type glutenin subunit. No significant differences in SDS sedimentation volume were noted between ‘Strongfield’, ‘AC Navigator’ and ‘WB881’. In all environments, the bread wheat checks had significantly higher SDS sedimentation volumes than all other genotypes evaluated (Table 4). This indicates that the bread wheat varieties had stronger gluten properties than the durum wheat genotypes used in this study.

4.1.6 Hagberg Falling Number (FN)

The combined analyses of variance (ANOVA) of means across environments detected significant differences in FN among the genotypes evaluated (Appendix A3). Averaged over all environments, the FN of ‘97Emmer19’ was similar to that of ‘Strongfield’ (Table 5). In contrast, ‘2000EB4’ showed significantly higher ($P<0.05$) FN values than ‘97Emmer19’ and the majority of emmer-derived breeding lines in all environments across years (Table 5). Averaged over all environments, no statistical differences ($P>0.05$) between ‘2000EB4’ and ‘WB881’ was noted (Table 5). In 2006, ‘05Emmereg-03’ and ‘05Emmereg-26’ consistently displayed higher FN than the other emmer-derived breeding lines ($P<0.05$), and were similar to ‘2000EB4’ (Table 5). In contrast, ‘05Emmereg-10’ had lower FN at the GDL and SF locations ($P<0.05$).

In 2005, the CIMMYT 1AS.1AL-1DL translocation lines had low FN compared to

Table 5. Least square means for Falling Number (sec) and Yellow Pigment Concentration (mg kg⁻¹) of bread wheat checks (AC Barrie, CDC Teal, and AC Superb) and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines) planted during the 2005 and 2006 growing seasons at Goodale (GDL), Kernen (KER) and Seed Farm (SF) locations.

Genotype	Falling number (seconds)							Yellow pigment concentration (mg kg ⁻¹)						
	GDL		KER		SF		C ^d	GDL		KER		SF		C
	'05 ^a	'06 ^b	'05	'06	'05	'06		'05	'06	'05	'06	'05	'06	
BREAD WHEAT CHECK CULTIVARS														
AC Barrie	414	416	416	438	426	486	433	3.3	3.1	2.7	2.5	4.0	2.4	3.0
CDC Teal	412	412	356	440	426	491	423	3.4	3.0	2.9	2.7	3.9	2.5	3.1
AC Superb	396	382	388	392	398	407	394	3.3	3.1	3.0	2.7	4.2	2.7	3.2
1AS.1AL-1DL TRANSLOCATION LINES														
04EDUYT-42	296	-	189	-	272	-	-	7.9	-	7.8	-	8.6	-	-
04EDUYT-43	331	-	153	-	287	-	-	7.7	-	7.4	-	8.6	-	-
04IDSN-107	304	-	189	-	278	-	-	8.1	-	7.6	-	8.7	-	-
04IDSN-111	330	-	145	-	292	-	-	8.1	-	7.6	-	8.9	-	-
L092	299	369	308	368	350	458	359	5.9	5.5	5.2	5.6	6.2	5.0	5.6
L252	248	336	287	356	314	398	323	5.7	5.3	4.8	5.3	6.1	4.5	5.3
S99B33	271	359	318	369	350	-	-	6.6	5.4	5.1	5.4	6.2	-	-
S99B34	275	363	327	364	352	384	344	6.1	5.6	5.1	5.4	6.2	4.8	5.5
DURUM WHEAT CULTIVARS														
Strongfield	289	405	299	433	329	435	365	7.8	7.7	6.9	7.0	8.5	6.4	7.4
WB881	351	434	360	480	411	533	428	6.7	6.8	5.9	6.4	7.6	6.0	6.6
Commander	308	431	280	456	421	539	406	7.9	7.9	7.4	7.3	8.9	7.2	7.8
AC Navigator	274	421	275	442	377	480	378	7.4	7.3	6.9	7.2	8.2	6.8	7.3
DT724	422	430	337	496	417	464	428	7.3	6.9	6.5	6.4	8.1	5.9	6.9
Stewart-63	275	332	196	380	350	388	320	5.4	4.8	5.1	4.5	6.1	4.5	5.1
Arcola	321	411	399	446	399	512	415	6.4	6.6	5.2	6.3	7.1	6.0	6.3
'97EMMER19' AND EMMER-DERIVED BREEDING LINES														
97Emmer19	326	446	271	444	228	531	374	4.8	5.2	4.1	4.4	5.4	4.1	4.7
2000EB4	446	474	401	493	433	571	470	5.9	5.9	4.9	5.4	6.6	5.1	5.7
X.98.142.17	360	360	308	403	342	405	363	7.4	5.9	5.9	5.2	7.3	5.2	6.1
X.98.142.18	341	-	333	-	382	-	-	5.7	-	5.0	-	6.8	-	-
P.01.64.31	247	388	233	449	334	455	351	6.8	6.5	5.9	6.3	7.2	5.7	6.4
P.01.64.39	254	403	280	476	372	537	387	7.4	7.3	6.7	7.3	8.1	6.5	7.2
P.01.64.62	275	399	310	441	339	497	377	7.8	7.7	7.1	7.3	7.1	7.3	7.5
05Emmereg-01	-	368	-	427	-	469	-	-	7.2	-	7.2	-	6.5	-
05Emmereg-03	-	434	-	500	-	554	-	-	7.0	-	6.2	-	6.1	-
05Emmereg-10	-	345	-	402	-	391	-	-	6.9	-	6.3	-	6.1	-
05Emmereg-26	-	394	-	454	-	634	-	-	7.1	-	7.0	-	6.7	-
LSD _{0.05} ^c	55	43	42	36	43	62	76	0.8	0.4	0.4	0.3	0.5	0.4	0.5

^a'05 = 2005 growing season

^b'06 = 2006 growing season

^cLSD = Least significant difference

^dC = Combined data across environments (GDL, KER, and SF) and/or growing seasons (2005 and 2006)

the bread wheat and durum wheat check cultivars ($P < 0.05$) (Table 5). At the KER location, '04IDSN-111' (145 sec) showed significantly lower FN value than '04EDUYT-42' and '04IDSN-107' ($P < 0.05$), although no significant difference was observed between '04IDSN-111' and '04EDUYT-43' (Table 5). The FN of 'Stewart-63' was similar to that of the CIMMYT translocation lines at GDL and KER in 2005, and significantly lower ($P < 0.05$) than 'Strongfield' in 2006 (Table 5). The USDA-ARS translocation lines had significantly lower FN than the bread wheat checks ($P < 0.05$), and among these lines, no significant differences were noted except at the SF location in 2006 (Table 5). At the SF in 2006, 'S99B34' showed a significantly lower FN value (384 sec) than 'L092' (458 sec) ($P < 0.05$), although no significant difference was observed between 'S99B34' and 'L252' (398 sec) (Table 5).

Compared to 'AC Barrie' and 'CDC Teal', 'Strongfield' had lower FN in all environments, but the differences were only significant ($P < 0.05$) in 2005. With the exception of the KER (2005) environment, 'AC Superb' showed consistently lower FN than 'AC Barrie' and 'CDC Teal', but differences were only significant ($P < 0.05$) at KER (2006) and SF (2006) (Table 5). Of the durum wheat checks, 'WB881' and 'DT724' had the highest FN ($P < 0.05$), and were similar to 'AC Barrie' and 'CDC Teal'. However at GDL and KER in 2005, where FN differences were most manifested (as evidenced by a larger range in FN), only 'DT724' was similar to 'CDC Teal' and 'AC Barrie' (Table 5).

4.1.7 Yellow Pigment Concentration (YPC)

For individual environments, the ANOVA revealed the genotype effect was statistically significant ($P < 0.01$) for YPC (Appendix A4). The variance estimate for the genotype x year x location interaction was not statistically significant ($P < 0.05$) Appendix A5). Correlations between YPC LS means among environments were high, and ranged from 0.94 ($P < 0.001$) to 0.98 ($P < 0.001$).

'97Emmer19' had low YPC and emmer breeding line '2000EB4' had lower YPC compared to the durum wheat checks ($P < 0.05$), including 'WB881' (Table 5). In contrast, emmer derived breeding lines 'P.01.64.39' and 'P.01.64.62' had similar YPC to 'Strongfield', which is the result of the breeding effort to increase YPC in these lines. Likewise, '05Emmereg-01' and '05Emmereg-26' had elevated YPC (Table 5) compared to their parent '2000EB4' ($P < 0.05$) (Table 1).

All of the USDA-ARS translocation lines had significantly lower ($P < 0.05$) YPC than the durum wheat checks in all environments. In contrast, in 2005, the CIMMYT translocation lines had similar YPCs to 'Commander' in all three environments (Table 5). Averaged over all environments, 'WB881', 'DT724', 'Stewart-63' and 'Arcola' had significantly lower YP than 'Strongfield', 'Commander' and 'AC Navigator' ($P < 0.05$). Of the durum wheat checks, 'Commander' had the highest YPC ($P < 0.05$), but was not significantly different from 'AC Navigator' and 'Strongfield' (Table 5). The higher YPCs of these newer varieties is due to the strong selection pressure for elevated pigment levels in Canadian durum wheat breeding programs (Clarke et al., 1998). As expected, the bread wheat varieties had lower yellow pigment than all of the durum wheat check cultivars ($P < 0.05$) in all environments.

4.2 Quality Evaluation of Composite Samples

CIMMYT lines had significantly higher YPC's than most of the tetraploid wheats tested ($P < 0.05$), but showed poor SDS Sedimentation volumes and lower GPC's ($P < 0.05$). Hence the best CIMMYT line (04EDUYT-43) out of four was considered for further milling and rheological quality tests, to compare the baking performance with other tetraploid genotypes included in this study.

Because of the large sample sizes required for triplicate analysis of rheological and baking quality tests, composite samples were produced within years. As such, years are the effective replication measuring environmental variation, and means comparisons between varieties is restricted to the combined analysis. Because repeated analyses of the composite samples (subsamples) were evaluated, the genotype x year x subsample interaction could be considered in the statistical model as the residual variation to test the variance estimate of the genotype x year interaction. Data is presented for each year, along with standard deviations of duplicate tests, providing a measure of precision for each quality assessment. As such, comparisons of genotypes within each year should be interpreted with caution, especially if the year x genotype interaction effect is significant.

4.2.1 Flour/Semolina Milling Yield and Flour/Semolina Protein Concentration (FP/SP)

Within years, triplicate millings were variable, with average standard deviations of 1.3% (range 0.3 - 2.8% for 2005) and 1.4% (range 0.3 - 3.2% for 2006) which was higher

Table 6. Mean and standard deviation of bread wheat checks (AC Barrie, CDC Teal, and AC Superb) and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines) for flour/semolina milling yield (%), flour/semolina protein concentration (14.0% mb), and agtron colour (%).

Genotype	FY/SY ^a			FP/SP ^b			Agtron ^c		
	2005 ^d	2006 ^e	C ^f	2005	2006	C	2005	2006	C
BREAD WHEAT CHECK CULTIVARS									
AC Barrie	72.6 ± 1.2	74.0 ± 0.6	73.3	13.9 ± 0.0	14.5 ± 0.0	14.2	65 ± 2	54 ± 1	59
CDC Teal	71.8 ± 0.9	73.5 ± 0.3	72.7	14.4 ± 0.0	14.5 ± 0.2	14.5	65 ± 2	55 ± 1	60
AC Superb	70.8 ± 0.9	73.9 ± 0.5	72.3	13.4 ± 0.0	13.7 ± 0.0	13.6	54 ± 3	49 ± 2	52
1AS.1AL-1DL TRANSLOCATION LINES									
04EDUYT-43	64.3 ± 2.5	-	-	9.8 ± 0.0	-	-	72 ± 1	-	-
L092	64.0 ± 2.8	64.5 ± 3.2	64.2	12.7 ± 0.0	12.3 ± 0.0	12.5	74 ± 2	79 ± 2	76
L252	63.5 ± 1.9	65.0 ± 1.9	64.3	13.6 ± 0.1	13.1 ± 0.0	13.4	66 ± 2	70 ± 2	68
S99B33	65.3 ± 1.1	64.4 ± 2.3	64.8	13.1 ± 0.0	12.7 ± 0.1	12.9	70 ± 1	77 ± 2	74
S99B34	63.9 ± 1.9	64.6 ± 1.2	64.3	13.0 ± 0.0	12.3 ± 0.0	12.7	74 ± 1	76 ± 2	75
DURUM WHEAT CULTIVARS									
Strongfield	63.5 ± 1.3	64.2 ± 0.8	63.8	13.0 ± 0.0	12.3 ± 0.1	12.6	70 ± 2	70 ± 2	70
WB881	65.0 ± 1.5	65.2 ± 1.2	65.1	12.1 ± 0.0	11.8 ± 0.0	11.9	74 ± 4	79 ± 2	76
Commander	64.2 ± 1.7	65.2 ± 2.0	64.7	11.4 ± 0.0	11.7 ± 0.0	11.6	67 ± 2	72 ± 2	69
AC Navigator	65.1 ± 0.6	65.7 ± 2.5	65.4	11.6 ± 0.0	12.1 ± 0.0	11.9	69 ± 1	74 ± 3	71
DT724	65.0 ± 1.3	64.2 ± 1.6	64.7	12.1 ± 0.0	12.1 ± 0.0	12.1	66 ± 2	74 ± 2	70
Stewart-63	64.5 ± 1.2	65.2 ± 0.9	64.9	11.9 ± 0.0	11.4 ± 0.0	11.7	79 ± 2	85 ± 2	82
Arcola	65.7 ± 0.3	65.6 ± 0.5	65.6	12.4 ± 0.0	11.9 ± 0.2	12.2	72 ± 2	78 ± 3	75
‘97EMMER19’ AND EMMER-DERIVED BREEDING LINES									
97Emmer19	62.3 ± 1.5	67.5 ± 1.5	64.9	13.2 ± 0.0	12.9 ± 0.0	13.0	48 ± 3	58 ± 2	53
2000EB4	64.6 ± 1.4	66.2 ± 1.0	65.4	12.9 ± 0.0	12.5 ± 0.0	12.7	62 ± 2	71 ± 2	67
X.98.142.17	65.7 ± 1.6	67.2 ± 0.9	66.4	11.7 ± 0.0	11.8 ± 0.1	11.8	67 ± 3	72 ± 1	69
X.98.142.18	66.4 ± 0.5	-	-	11.5 ± 0.1	-	-	74 ± 1	-	-
P.01.64.31	66.2 ± 1.7	66.6 ± 2.1	66.4	11.6 ± 0.0	11.4 ± 0.0	11.5	72 ± 2	82 ± 2	77
P.01.64.39	66.1 ± 0.7	65.2 ± 0.7	65.7	13.0 ± 0.0	13.0 ± 0.1	13.0	61 ± 2	74 ± 2	68
P.01.64.62	65.1 ± 1.0	65.3 ± 1.8	65.2	11.6 ± 0.0	11.3 ± 0.0	11.4	62 ± 1	70 ± 1	66
05Emmerreg-01	-	64.7 ± 1.9	-	-	10.6 ± 0.0	-	-	81 ± 2	-
05Emmerreg-03	-	62.6 ± 1.1	-	-	13.8 ± 0.0	-	-	76 ± 2	-
05Emmerreg-10	-	65.7 ± 2.6	-	-	12.1 ± 0.0	-	-	71 ± 2	-
05Emmerreg-26	-	66.5 ± 2.1	-	-	12.2 ± 0.0	-	-	76 ± 1	-
LSD ^g _{0.05}			2.1			0.6			9

Values represent mean ± standard deviation of triplicate (FY/SY) or duplicate (FP/SP and Agtron) determinations

^aFY/SY = flour/semolina milling yield (%)

^bFP/SP = Flour/Semolina protein concentration (14.0% moisture basis)

^cAgtron = Agtron colour (%)

^d2005 = 2005 growing season

^e2006 = 2006 growing season

^fC = Combined composite samples data across years (2005 and 2006)

^gLSD = Least Significant Difference

than expected. Generally, there was less variation in triplicate millings of bread wheats compared to durum wheat genotypes (Table 6). The durum wheat milling method described in this thesis, however, is not a four stand Allis-Chalmers mill (used for durum milling at GRL, Winnipeg, Manitoba) for determining semolina yield, and uses considerably less sample than does the GRL, which could explain the larger variation observed in triplicate millings. The emmer-derived breeding lines 'X.98.142.17' and 'P.01.64.31' showed significantly higher semolina yield than did the durum wheat check 'Strongfield' ($P < 0.05$) (Table 6). Averaged over both years, the bread wheat check cultivars had higher milling yields than all of the durum wheat varieties evaluated ($P < 0.05$).

For FP/SP, ANOVA revealed significant genotypic effects (Appendix A5). Analyses of FP/SP were highly reproducible as evidenced by the low standard deviations of duplicate samples (Table 6). In both 2005 and 2006 composite samples, the majority of emmer-derived breeding lines had lower SP than 'Strongfield', and was numerically similar to 'Commander'. Only '2000EB4' and 'P.01.64.39' had SP similar to 'Strongfield', which was consistent with the whole meal GPC data (Table 6). 'L252' had significantly higher SP than the other USDA-ARS translocation lines ($P < 0.05$) evaluated and statistically higher SP than 'Strongfield' ($P < 0.05$) (Table 6). Averaged over both years, 'Strongfield' had higher SP than 'WB881', 'Commander' and 'AC Navigator' ($P < 0.05$), but was similar to '97Emmer19'. Based on repeated evaluation in registration trials conducted in western Canada, 'Commander' and 'AC Navigator' are known to have approx. 0.5% - 1% less protein than 'Strongfield' (Clarke et al., 2006a; Clarke et al., 2001a; Clarke et al., 2006b). Similar to whole meal protein analysis (Results section 4.1.4), the FP was higher in bread wheats than in durum wheat varieties, '97Emmer19' and the emmer-derived breeding lines.

Comparison of the LS means from the combined analysis for whole meal (Table 4) and FP/SP (Table 6) revealed protein losses were greater in durum vs. bread wheat varieties ($P < 0.05$). This is likely due to the lower semolina extraction rates observed for durum wheats compared to the flour extraction rates for the bread wheats (Table 6). Lower extraction rates are expected to result in increased protein losses due primarily to

greater loss of peripheral, higher protein endosperm, and to a lesser degree, more complete removal of high protein aleurone tissue.

4.2.2 Flour/Semolina Colour

The colour of flour and semolina is an important criterion for both pasta and bread quality. Colour of the flour and semolina was assessed using both an Agtron and a Hunterlab Miniscan (CIELab colour space) colorimeter, two commonly used methods for colour assessment in breeding programs and in the milling industry.

For combined analyses of variance (ANOVA) of both 2005- and 2006- grown genotypes, genotypes differed in flour/semolina Agtron colour (Appendix A5). Excluding bread wheat checks, the Agtron colour values of 2006 composite samples were higher than those of 2005 composite samples and the results were reproducible given the low duplicate sample standard deviations (Table 6). The Agtron colour of '97Emmer19' was not statistically different ($P < 0.05$) from the bread wheat checks, and significantly lower than all durum wheat genotypes tested ($P < 0.05$). All of the emmer-derived breeding lines evaluated in both 2005 and 2006, had significantly higher ($P < 0.05$) Agtron colour values than '97Emmer19' and were similar to 'Strongfield' and 'Commander' (Table 6). In 2006, 'P.01.64.31' and '05Emmerreg-01' had numerically (statistically non-significant) higher Agtron colour than 'Strongfield' and were similar to 'WB881' and 'Stewart-63' (Table 6). Among the durum and emmer-derived breeding lines, semolina Agtron values were similar for the Canadian varieties 'Strongfield', 'Commander' and 'AC Navigator'. 'Stewart-63' had a significantly higher Agtron value than most durum wheat genotypes evaluated ($P < 0.05$). Averaged over both composite samples, the bread wheat checks 'AC Barrie', 'CDC Teal', and 'AC Superb' had lower Agtron scores compared to the durum wheat genotypes evaluated ($P < 0.05$) (Table 6). 'AC Superb' showed numerically lower Agtron value than 'CDC Teal' and 'AC Barrie', suggesting poorer colour relative to the other two bread wheat checks.

The ANOVA for CIELab data for L^* , a^* and b^* is presented in Appendix A5. For all three measurements, significant ($P < 0.05$) genotypic effects were detected for L^* and a^* , although the variance estimates for the two-way interaction between genotype and year was not significantly different from zero ($P > 0.05$) (Appendix A5). These results suggest that the relative differences between genotypes were consistent between yearly

Table 7. Mean and standard deviation of bread wheat checks (AC Barrie, CDC Teal, and AC Superb) and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines) for flour/semolina brightness (L*), redness (a*), and yellowness (b*).

Genotype	L* ^a			a* ^b			b* ^c		
	2005 ^d	2006 ^e	C ^f	2005	2006	C	2005	2006	C
BREAD WHEAT CHECK CULTIVARS									
AC Barrie	85.1 ± 0.2	85.7 ± 0.2	85.4	1.21 ± 0.01	1.49 ± 0.02	1.35	12.9 ± 0.0	14.5 ± 0.0	13.7
CDC Teal	85.2 ± 0.4	85.9 ± 0.1	85.5	1.04 ± 0.02	1.28 ± 0.01	1.16	12.6 ± 0.0	14.8 ± 0.0	13.7
AC Superb	84.1 ± 0.4	85.1 ± 0.2	84.6	1.39 ± 0.06	1.60 ± 0.05	1.49	12.8 ± 0.0	14.6 ± 0.1	13.7
1AS.1AL-1DL TRANSLOCATION LINES									
04EDUYT-43	86.1 ± 0.3	-	-	0.90 ± 0.05	-	-	24.4 ± 0.1	-	-
L092	86.2 ± 0.2	86.5 ± 0.3	86.3	0.73 ± 0.03	0.87 ± 0.04	0.80	21.9 ± 0.0	23.6 ± 0.1	22.8
L252	85.7 ± 0.3	86.1 ± 0.2	85.9	0.61 ± 0.04	0.83 ± 0.06	0.72	20.6 ± 0.0	22.1 ± 0.1	21.3
S99B33	85.9 ± 0.3	86.4 ± 0.2	86.2	0.74 ± 0.06	0.91 ± 0.07	0.82	21.8 ± 0.1	23.4 ± 0.1	22.6
S99B34	86.2 ± 0.2	86.5 ± 0.2	86.3	0.73 ± 0.01	0.89 ± 0.04	0.81	21.9 ± 0.1	23.5 ± 0.1	22.7
DURUM WHEAT CULTIVARS									
Strongfield	85.5 ± 0.2	85.9 ± 0.3	85.7	1.08 ± 0.03	1.18 ± 0.06	1.13	24.7 ± 0.4	26.0 ± 0.0	25.3
WB881	86.1 ± 0.3	86.5 ± 0.3	86.3	0.77 ± 0.06	0.91 ± 0.06	0.84	23.6 ± 0.0	25.4 ± 0.1	24.5
Commander	85.3 ± 0.2	85.7 ± 0.3	85.5	1.18 ± 0.06	1.29 ± 0.08	1.24	27.1 ± 0.1	28.4 ± 0.0	27.8
AC Navigator	85.5 ± 0.2	86.0 ± 0.3	85.8	1.04 ± 0.04	1.23 ± 0.06	1.13	25.2 ± 0.1	26.1 ± 0.1	25.7
DT724	85.5 ± 0.3	86.0 ± 0.3	85.8	0.87 ± 0.06	0.93 ± 0.09	0.90	23.8 ± 0.1	24.6 ± 0.2	24.2
Stewart-63	87.2 ± 0.1	87.6 ± 0.2	87.4	0.62 ± 0.02	0.71 ± 0.04	0.66	17.3 ± 0.4	18.1 ± 0.2	17.7
Arcola	86.3 ± 0.3	86.7 ± 0.3	86.5	0.70 ± 0.06	0.87 ± 0.10	0.79	21.5 ± 0.0	22.9 ± 0.0	22.2
'97EMMER19' AND EMMER-DERIVED BREEDING LINES									
97Emmer19	84.9 ± 0.2	85.7 ± 0.3	85.3	1.37 ± 0.05	1.51 ± 0.04	1.44	15.1 ± 0.0	15.1 ± 0.1	15.1
2000EB4	85.4 ± 0.2	86.2 ± 0.2	85.8	0.72 ± 0.04	0.93 ± 0.06	0.82	20.7 ± 0.0	21.4 ± 0.0	21.1
X.98.142.17	85.5 ± 0.4	86.3 ± 0.2	85.9	1.04 ± 0.08	1.06 ± 0.02	1.05	20.5 ± 0.3	21.5 ± 0.1	21.0
X.98.142.18	86.3 ± 0.2	-	-	0.69 ± 0.08	-	-	21.2 ± 0.2	-	-
P.01.64.31	85.9 ± 0.2	86.7 ± 0.2	86.3	0.82 ± 0.07	0.81 ± 0.06	0.81	22.8 ± 0.0	24.6 ± 0.0	23.7
P.01.64.39	84.9 ± 0.3	85.9 ± 0.2	85.4	1.03 ± 0.08	1.24 ± 0.07	1.14	24.2 ± 0.0	26.6 ± 0.0	25.4
P.01.64.62	84.8 ± 0.2	85.5 ± 0.2	85.2	1.21 ± 0.06	1.31 ± 0.05	1.26	25.9 ± 0.0	27.6 ± 0.1	26.7
05Emmereg-01	-	86.8 ± 0.2	-	-	0.87 ± 0.05	-	-	25.5 ± 0.0	-
05Emmereg-03	-	86.5 ± 0.2	-	-	0.84 ± 0.01	-	-	23.9 ± 0.4	-
05Emmereg-10	-	85.7 ± 0.2	-	-	1.02 ± 0.11	-	-	25.9 ± 0.1	-
05Emmereg-26	-	86.1 ± 0.2	-	-	1.07 ± 0.01	-	-	25.1 ± 0.2	-
LSD ^g _{0.05}			0.4			0.11			0.9

Values represent mean ± standard deviation of duplicate determinations

^aL* = flour/semolina brightness

^ba* = flour/semolina redness

^cb* = flour/semolina yellowness

^d2005 = 2005 growing season

^e2006 = 2006 growing season

^fC = Combined composite samples data across years (2005 and 2006)

^gLSD = Least Significant Difference

composite samples and that for duplicate samples, the L* and a* values were reproducible. The direct comparisons of CIELab measurements L*, a* and b* between bread wheat and durum genotypes are not appropriate since the emmer-derived breeding lines were selected for superior semolina yellowness (b*) compared to their durum parents.

The flour/semolina L* values of 2006 composite samples were greater than those of 2005 composite samples (Table 7). Of the emmer-derived breeding lines, 'P.01.64.31' had a significantly higher ($P < 0.05$) L* (86.3) than all other emmer-derived breeding lines, although no significant difference was seen between 'P.01.64.31' and 'X.98.142.17' (85.9) (Table 7). The USDA-ARS 1AS.1AL-1DL translocation lines showed no significant flour/semolina brightness (L*) differences with the data averaged over yearly composite samples (Table 7). 'Stewart-63' (87.4) showed significantly higher semolina brightness than other durum genotypes ($P < 0.05$) (Table 7). 'Commander' (85.5) showed significantly lower semolina brightness than 'WB881' (86.3) and 'Arcola' (86.5) ($P < 0.05$), although no significant difference was detected between 'Commander', 'Strongfield' (85.7), 'AC Navigator' (85.8) and 'DT 724' (85.8) (Table 7).

The semolina redness values (a*) were higher in 2006 composite samples (Table 7). '97Emmer19' had the highest a* of all genotypes tested, but '2000EB4' had a significantly lower a* ($P < 0.05$), similar to its parent 'WB881' (Table 7). 'P.01.64.39' and 'P.01.64.62' had a* values statistically ($P > 0.05$) similar to 'AC Navigator'. These results were not surprising given that these lines are derived from a backcross of 'AC Navigator' to '2000EB4' (Table 1). All of the 05Emmereg series lines evaluated in 2006 also displayed lower a* values than '97Emmer19' and 'Commander' (Table 7). The combined analysis revealed that 'Commander' has a significantly higher a* than the other durum wheat genotypes except 'AC Navigator' ($P < 0.05$) which is consistent with previously reported results (Clarke et al., 2006a). There were no significant differences ($P < 0.05$) between the remaining durum wheat checks. When comparing bread wheat check cultivars, 'AC Superb' had statistically higher a* than 'CDC Teal' and 'AC Barrie' ($P < 0.05$). 'CDC Teal' was also noted to have reduced a* compared to 'AC Barrie' ($P < 0.05$), and these results were consistent between yearly composite samples (Table 7).

Semolina b* values were highly variable, and ranged from 15.1 for ‘97Emmer19’, to 27.8 for ‘Commander’ (Table 7). Of the emmer-derived breeding lines, ‘P.01.64.39’ and ‘P.01.64.62’ had b* value similar to their parent ‘AC Navigator’, and this similarity was consistent across yearly composite samples (Table 7). The USDA-ARS translocation lines had significantly higher b* than ‘97Emmer19’ (P<0.05), and were statistically similar to ‘Arcola’ (Table 7). Statistically, ‘Commander’ had the highest semolina b* of the durum wheat genotypes with the exception of emmer-derived breeding line ‘P.01.64.62’ (Table 7). ‘Stewart-63’ displayed poor semolina yellowness compared to the durum wheat check cultivars. Correlation coefficients for composite sample b* versus LS means for YP (Table 5) were 0.96 (P<0.01) and 0.95 (P<0.01) for 2005 and 2006, respectively, indicating that YPC is a good predictor of semolina colour in durum wheat.

4.2.3 Flour/Semolina Gluten Index (GI)

The ANOVA for GI indicated that the genotype and year effects were significant (P<0.05) with a strong genotypic effect (Appendix A5). ‘97Emmer19’ had a GI significantly lower than that of ‘Strongfield’ (P<0.05). In contrast, four of the five emmer-derived breeding lines evaluated over both years had GI values greater than ‘Commander’, but only ‘2000EB4’ was statistically higher (P<0.05). ‘Commander’ is classified as an extra-strong gluten type (Clarke et al., 2006a), and the higher SDS sedimentation volumes relative to ‘Strongfield’ are likely associated with increased gluten strength (Table 8) as SDS sedimentation volume is an effective indicator of gluten strength (Dexter et al., 1981; Dessalegn et al., 2006). In this study, the GI data was positively correlated (r = 0.81; P<0.01) with SDS Sedimentation volumes (Tables 14 and 15). All of the USDA-ARS substitution lines had GI values >98% (Table 8), which was statistically greater than ‘AC Superb’, ‘Commander’ and ‘WB881’ (P<0.05). ‘L252’ is known to carry the LWM-1 glutenin subunit (Klindworth et al., 2005) like ‘Stewart-63’. However, the GI of ‘Stewart-63’ was only 1%, and confirms the results of SDS sedimentation volumes (Table 4) that the 1AS.1AL-1DL substitution compensates for the reduced gluten strength conferred by the LMW-1 glutenin subunit.

The durum wheats ‘Commander’ and ‘WB881’, both extra-strong gluten types, had high GI values which were not significantly different from the bread wheat checks (Table 7). ‘DT724’ (P>0.05) showed lower GI than the bread wheats (P<0.05), but was

Table 8. Mean and standard deviation of bread wheat checks (AC Barrie, CDC Teal, and AC Superb) and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines) for flour/semolina gluten index (%), wet gluten content (%), and dry gluten content (%).

Genotype	GI ^a			WG ^b			DG ^c		
	2005 ^d	2006 ^e	C ^f	2005	2006	C	2005	2006	C
BREAD WHEAT CHECK CULTIVARS									
AC Barrie	98 ± 0.0	89 ± 1.3	94	38.5 ± 0.4	41.7 ± 0.4	40.1	13.6 ± 0.3	14.5 ± 0.2	14.0
CDC Teal	96 ± 1.7	93 ± 2.8	95	39.0 ± 0.4	39.8 ± 0.6	39.4	13.6 ± 0.1	13.8 ± 0.2	13.7
AC Superb	97 ± 2.2	89 ± 0.2	93	36.0 ± 2.0	37.5 ± 0.7	36.8	12.6 ± 0.8	13.0 ± 0.3	12.8
1AS.1AL-1DL TRANSLOCATION LINES									
04EDUYT-43	21 ± 1.7	-	-	21.7 ± 0.4	-	-	7.7 ± 0.3	-	-
L092	98 ± 1.2	98 ± 0.2	98	28.9 ± 0.1	30.2 ± 0.5	29.5	10.6 ± 0.0	10.9 ± 0.4	10.7
L252	98 ± 0.6	98 ± 1.2	98	33.8 ± 0.1	33.8 ± 0.1	33.8	11.9 ± 0.0	11.6 ± 0.1	11.7
S99B33	98 ± 0.6	100 ± 0.1	99	29.8 ± 0.4	32.1 ± 0.2	30.9	11.0 ± 0.4	11.1 ± 0.1	11.1
S99B34	99 ± 0.2	99 ± 0.0	99	29.2 ± 0.1	30.7 ± 1.0	30.0	11.2 ± 0.1	11.1 ± 0.5	11.1
DURUM WHEAT CULTIVARS									
Strongfield	61 ± 0.4	62 ± 1.2	62	36.8 ± 0.0	36.5 ± 0.0	36.7	13.2 ± 0.2	13.0 ± 0.1	13.1
WB881	94 ± 3.3	84 ± 1.4	89	33.7 ± 0.4	34.6 ± 0.1	34.2	12.0 ± 0.1	12.2 ± 0.1	12.1
Commander	92 ± 2.9	86 ± 2.8	89	31.8 ± 0.3	34.0 ± 0.0	32.9	11.3 ± 0.3	11.8 ± 0.1	11.6
AC Navigator	67 ± 3.4	64 ± 1.2	65	33.5 ± 0.3	37.7 ± 0.3	35.6	11.8 ± 0.1	13.4 ± 0.3	12.6
DT724	81 ± 0.1	83 ± 0.2	82	38.6 ± 0.6	35.4 ± 0.4	37.0	12.0 ± 0.1	12.5 ± 0.2	12.2
Stewart-63	1 ± 0.3	1 ± 0.2	1	29.0 ± 2.9	36.4 ± 0.8	32.7	10.5 ± 1.1	10.4 ± 0.1	10.5
Arcola	64 ± 1.7	55 ± 0.2	59	37.1 ± 0.8	37.0 ± 0.3	37.1	13.8 ± 0.6	13.4 ± 0.3	13.6
‘97EMMER19’ AND EMMER-DERIVED BREEDING LINES									
97Emmer19	46 ± 1.5	42 ± 0.0	44	39.4 ± 0.7	41.7 ± 0.6	40.5	14.1 ± 0.1	14.6 ± 0.3	14.4
2000EB4	98 ± 0.4	94 ± 3.3	96	35.2 ± 0.3	36.3 ± 0.4	35.8	12.4 ± 0.0	12.8 ± 0.1	12.6
X.98.142.17	97 ± 1.4	93 ± 2.2	95	33.1 ± 0.4	35.3 ± 0.1	34.2	11.9 ± 0.1	12.5 ± 0.1	12.2
X.98.142.18	95 ± 2.2	-	-	32.0 ± 0.9	-	-	11.6 ± 0.7	-	-
P.01.64.31	92 ± 3.6	92 ± 1.3	92	33.7 ± 0.7	33.3 ± 0.1	33.5	11.9 ± 0.4	11.7 ± 0.0	11.8
P.01.64.39	80 ± 3.6	74 ± 4.1	77	36.9 ± 0.1	39.2 ± 0.2	38.0	13.1 ± 0.1	13.7 ± 0.0	13.4
P.01.64.62	97 ± 0.3	86 ± 0.6	92	32.6 ± 0.4	34.1 ± 0.1	33.3	11.5 ± 0.1	11.8 ± 0.3	11.6
05Emmereg-01	-	70 ± 1.3	-	-	31.8 ± 0.1	-	-	11.2 ± 0.1	-
05Emmereg-03	-	59 ± 0.2	-	-	41.5 ± 0.1	-	-	14.8 ± 0.1	-
05Emmereg-10	-	85 ± 1.9	-	-	35.5 ± 0.1	-	-	12.4 ± 0.1	-
05Emmereg-26	-	83 ± 4.1	-	-	36.1 ± 0.1	-	-	12.6 ± 0.0	-
LSD ^g _{0.05}			6			3.1			0.7

Values represent mean ± standard deviation of duplicate determinations

^aGI = flour/semolina gluten index

^bWG = flour/semolina wet gluten content

^cDG = flour/semolina dry gluten content

^d2005 = 2005 growing season

^e2006 = 2006 growing season

^fC = Combined composite samples data across years (2005 and 2006)

^gLSD = Least Significant Difference

not statistically different from ‘Commander’ or ‘WB881’. ‘Strongfield’ and ‘AC Navigator’ are both considered conventional strength durum wheat varieties (Clarke et al., 2006b; Clarke et al., 2001a), and exhibited ($P>0.05$) GI values of 62% and 65%, respectively. The bread wheat checks possessed high GI, averaging 94% (Table 8).

4.2.4 Flour/Semolina Wet (WG) and Dry (DG) Gluten

The ANOVA of 2005 and 2006 grown genotypes revealed significant differences among genotypes for both WG and DG (Appendix A5). The WG variance estimates for genotype x year were not statistically different from zero (Appendix A5), suggesting relative ranking of genotypes was consistent between yearly composite samples.

The WG content of ‘97Emmer19’ was significantly higher than that of the current durum wheat varieties ‘Strongfield’, ‘Commander’ and ‘AC Navigator’ ($P<0.05$) (Table 8). Of the emmer-derived breeding lines, only ‘P.01.64.39’ had WG similar to ‘97Emmer19’ with the WG of the remaining lines being similar to ‘Commander’, ‘AC Navigator’ and ‘WB881’ (Table 8). The USDA-ARS translocation lines had WG content ranging from 29.5% to 33.8% (Table 8). The WG content of bread wheat cultivars was higher than that of the majority of durum wheat cultivars (Table 8), and is consistent with earlier results (Pasha et al., 2007). However, ‘Strongfield’, ‘DT724’ and ‘Arcola’ had elevated WG content, similar to the bread wheat check ‘CDC Teal’.

Table 9. Correlation coefficients between LS means of flour/semolina protein concentration (%) and wet and dry gluten (%).

Quality Parameter	Wet Gluten (%)	Dry Gluten (%)
Flour/Semolina Protein (%)	0.50*	0.52*
Dry Gluten (%)	0.94**	

* $P < 0.05$ ** $P < 0.01$

Pasha et al (2007) found that both WG and DG traits were correlated with FP/SP, with correlation coefficients ranging from 0.68 ($P<0.01$) to 0.69 ($P<0.01$), confirming that these variables are dependent on protein concentration. The correlation between WG (%) and DG (%) was 0.94 ($P<0.01$) (Table 9). However, examination of the deviation of genotype LS means from the covariance estimate revealed that ‘Stewart-63’ explained a large portion of the covariance deviation, with lower than expected DG relative to its WG

Table 10. Farinograph quality parameters (calculated from farinograph curves) of bread wheat checks (AC Barrie, CDC Teal, and AC Superb) and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines).

Genotype	FAB ^a			STA ^b			MTI ^c			DDT ^d			TTB ^e		
	'05 ^f	'06 ^g	C ^h	'05	'06	C	'05	'06	C	'05	'06	C	'05	'06	C
BREAD WHEAT CHECK CULTIVARS															
AC Barrie	63.0	62.4	62.7	13.5	23.8	18.7	30	20	25	7.0	7.5	7.3	13.0	11.5	12.3
CDC Teal	64.2	63.2	63.7	22.0	37.0	29.5	20	10	15	10.0	7.5	8.8	18.0	17.0	17.5
AC Superb	65.0	63.6	64.3	20.0	19.2	19.6	25	30	28	6.5	5.2	5.9	10.0	8.5	9.3
1AS.1AL-1DL TRANSLOCATION LINES															
04EDUYT-43	60.4	-	-	3.5	-	-	105	-	-	2.0	-	-	4.5	-	-
L092	60.0	58.4	59.2	26.0	50.0	38.0	15	10	13	18.5	17.5	18.0	21.0	43.0	32.0
L252	62.2	59.8	61.0	29.0	26.0	27.5	20	30	25	12.0	10.5	11.3	22.0	15.5	18.8
S99B33	62.0	60.0	61.0	34.5	50.2	42.4	10	5	8	15.5	14.0	14.8	26.5	29.0	27.8
S99B34	61.2	58.2	59.7	41.5	36.3	38.9	20	10	15	15.0	11.0	13.0	27.0	38.0	32.5
DURUM WHEAT CULTIVARS															
Strongfield	60.0	55.8	57.9	7.2	6.2	6.7	55	40	48	1.8	2.2	2.0	4.0	5.0	4.5
WB881	60.0	57.2	58.6	10.0	6.7	8.4	40	45	43	4.5	3.2	3.9	7.0	6.5	6.8
Commander	61.6	59.4	60.5	6.2	7.3	6.8	40	40	40	2.5	3.7	3.1	5.0	5.5	5.3
AC Navigator	62.4	59.8	61.1	5.0	3.6	4.3	40	50	45	3.0	2.5	2.8	4.0	4.3	4.2
DT724	61.8	59.6	60.7	7.0	6.8	6.9	40	35	38	4.5	3.2	3.9	6.5	7.0	6.8
Stewart-63	60.6	57.6	59.1	2.5	1.4	2.0	90	105	98	2.0	1.6	1.8	4.5	2.5	3.5
Arcola	58.0	55.2	56.6	3.7	3.5	3.6	60	60	60	3.5	2.7	3.1	5.0	4.5	4.8
'97EMMER19' AND EMMER-DERIVED BREEDING LINES															
97Emmer19	57.8	54.8	56.3	3.7	3.1	3.4	80	85	83	2.8	2.3	2.6	3.5	3.2	3.4
2000EB4	60.0	57.6	58.8	25.0	22.0	23.5	20	30	25	8.0	6.0	7.0	15.0	10.0	12.5
X.98.142.17	58.6	57.2	57.9	8.5	8.2	8.4	40	40	40	5.0	4.5	4.8	8.5	8.0	8.3
X.98.142.18	59.6	-	-	11.0	-	-	30	-	-	6.0	-	-	9.0	-	-
P.01.64.31	61.2	56.8	59.0	8.5	7.5	8.0	40	30	35	3.5	5.0	4.3	7.0	9.5	8.3
P.01.64.39	63.6	60.0	61.8	6.5	7.9	7.2	40	55	48	3.0	3.0	3.0	7.0	5.0	6.0
P.01.64.62	60.6	57.4	59.0	13.8	19.4	16.6	30	25	28	5.5	3.5	4.5	9.0	9.0	9.0
05Emmerreg-01	-	55.2	-	-	6.7	-	-	60	-	-	3.0	-	-	5.0	-
05Emmerreg-03	-	61.2	-	-	11.2	-	-	50	-	-	3.5	-	-	5.0	-
05Emmerreg-10	-	58.6	-	-	11.0	-	-	30	-	-	4.7	-	-	8.5	-
05Emmerreg-26	-	57.2	-	-	7.7	-	-	40	-	-	3.5	-	-	6.5	-
LSD _{0.05} ⁱ			1.5			11.3			13			1.9			8.7

^aFAB = Farinograph water absorption (%) ^bSTA = Farinograph stability (min) ^cMTI = Farinograph Mixing Tolerance Index (B.U.) ^dDDT = Farinograph dough development time (min) ^eTTB = Farinograph time to breakdown (min) ^f'05 = 2005 growing season ^g'06 = 2006 growing season ^hC = Combined composite samples data across years (2005 and 2006) ⁱLSD = Least Significant Difference

content (Table 9). This could imply that the gluten of ‘Stewart-63’ has greater water hydration capacity, perhaps due to the presence of the LMW-1 glutenin subunit.

4.3 Rheological Properties of Semolina/Flour and Bake Test

4.3.1 Farinograph Assessment of Rheological Properties

The farinograph is used world-wide as a tool to determine water absorption potential of flour and semolina, and to assess dough rheological properties. For farinograph water absorption (FAB), farinograph stability (STA), farinograph mixing tolerance index (MTI), farinograph dough development time (DDT) and farinograph time to breakdown (TTB). Significant differences were detected among genotypes (Appendix A6).

‘97Emmer19’ had the lowest FAB (56.3%), and was statistically lower than ‘Strongfield’ ($P < 0.05$) but similar to ‘Arcola’ (Table 10). The emmer-derived genotypes evaluated over both years had similar FAB to ‘Stewart-63’ and were not statistically different from ‘Commander’ ($P > 0.05$). In 2006 composite samples, ‘05Emmereg-01’ had the lowest FAB compared to other emmer-derived breeding lines evaluated. On average, the durum wheat genotypes tested had statistically lower ($P < 0.05$) FAB than the bread wheat checks. ‘Strongfield’ and ‘Arcola’ had lower FAB than ‘Commander’, ‘AC Navigator’ and ‘DT724’ ($P < 0.05$) (Table 10). The FAB of hexaploid wheat entries ranged from 62.7% for ‘AC Barrie’ to 64.3% for ‘AC Superb’ (Table 10), but no significant differences were noted ($P > 0.05$) between the three genotypes. Apart from the high starch damage (Evans and Stevens, 1985; Tipples, 1969; Bass, 1988) and high flour protein concentration (Hruskova et al., 2006), the intrinsic differences in protein quantity and quality (Edwards et al., 1996) also results in greater water absorption during dough mixing and in this study the protein quality rather than quantity appears to be the reason for the increased FAB in bread wheat checks as compared to durum and emmer wheats.

Farinograph stability (STA) and the mixing tolerance index (MTI) are measures of the tolerance of a flour/semolina to mixing, and are related to the gluten properties of the flour/semolina. ‘97Emmer19’ showed a high MTI, not statistically different from ‘Stewart-63’. The STA (23.5 min) and MTI (25 B.U.) of ‘2000EB4’ was similar to those of the bread wheat checks and ‘L252’ (Table 10), although no significant difference was seen between ‘2000EB4’ and ‘P.01.64.62’ for STA and MTI parameters. Dough from the

USDA-ARS translocation lines, except 'L252', displayed STA above the bread wheat and durum wheat genotypes, with values ranging from 27.5 minutes to 42.4 minutes (Table 10), indicating very strong gluten. A corresponding low MTI was also noted for these lines (Table 10). 'Stewart-63', which carries the LMW-1 glutenin subunit, has a low STA, and statistically higher MTI than all other durum wheat genotypes ($P < 0.05$), indicating dough had poor tolerance to mixing (weak gluten). Among the bread wheat checks, the STA of 'CDC Teal' was higher than those of 'AC Barrie' and 'AC Superb' in both 2005 and 2006 composite samples, but the differences were not statistically significant (Table 10).

Farinograph dough development time (DDT), which measures relative strength (gluten strength) of the flour/semolina (Zounis and Quail, 1997), ranged from 1.8 min ('Stewart-63') to 18.0 min ('L092') in the genotypes evaluated (Table 10). The farinograph time to breakdown (TTB) values showed a similar trend as DDT (Table 10) with a correlation coefficient of 0.97 ($P < 0.001$) with DDT. All USDA-ARS translocation lines, except 'L252', displayed DDT and TTB values above the bread wheat and durum wheat genotypes (Table 10). 'Stewart-63', which carries the LMW-1 glutenin subunit, showed a statistically shorter ($P < 0.05$) DDT than most of the durum wheat genotypes, indicating weak dough strength.

4.3.2 Alveograph Assessment of Dough Rheology

The ANOVA for alveograph dough tenacity (P), alveograph extensibility (L), alveograph curve configuration ratio (P/L) and alveograph strength (W) for both the 2005- and 2006- grown genotypes are presented in Appendix A6. Significant genotypic effects were detected for all four alveograph parameters. Alveograph parameters are influenced by the extent of starch damage after milling and the water absorption capacity of the flour/semolina, with increasing starch damage resulting in reduced dough extensibility (L) and increased pressure (P) (Edwards and Dexter, 1987). Given the differences in hardness (and thus starch damage) between bread wheat and durum wheat (Table 3) and the differences in FAB (Table 10), comparison between bread wheat and durum wheat genotypes should be interpreted with caution.

Alveograph P values of tetraploid wheat genotypes were high, with a range of 39 mm to 161 mm (Table 11). The P value of '97Emmer19' was 39 mm, statistically lower

Table 11. Alveograph quality parameters (mean and standard deviation) of bread wheat checks, AC Barrie, CDC Teal, and AC Superb, and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines).

Genotype	P ^a			L ^b			P/L ^c			W ^d		
	2005 ^e	2006 ^f	C ^g	2005	2006	C	2005	2006	C	2005	2006	C
BREAD WHEAT CHECK CULTIVARS												
AC Barrie	91 ± 4	99 ± 6	95	130 ± 26	82 ± 9	106	0.74 ± 0.23	1.22 ± 0.11	0.98	419 ± 52	325 ± 32	372
CDC Teal	86 ± 9	89 ± 6	88	123 ± 27	110 ± 12	117	0.75 ± 0.29	0.82 ± 0.13	0.78	369 ± 59	343 ± 22	356
AC Superb	97 ± 3	85 ± 6	91	141 ± 11	88 ± 23	114	0.69 ± 0.06	1.01 ± 0.24	0.85	431 ± 25	259 ± 34	345
1AS.1AL-1DL TRANSLOCATION LINES												
04EDUYT-43	63 ± 1	-	-	48 ± 29	-	-	1.87 ± 1.25	-	-	67 ± 12	-	-
L092	164 ± 21	125 ± 12	145	30 ± 5	46 ± 7	38	5.65 ± 1.41	2.78 ± 0.56	4.21	194 ± 37	269 ± 41	231
L252	172 ± 9	109 ± 4	141	49 ± 6	62 ± 6	55	3.56 ± 0.57	1.79 ± 0.24	2.67	371 ± 36	293 ± 16	332
S99B33	185 ± 20	138 ± 2	161	29 ± 3	52 ± 7	41	6.38 ± 0.99	2.72 ± 0.48	4.55	243 ± 31	325 ± 38	284
S99B34	160 ± 19	132 ± 10	146	34 ± 9	46 ± 8	40	4.95 ± 1.35	2.92 ± 0.46	3.94	245 ± 84	286 ± 51	266
DURUM WHEAT CULTIVARS												
Strongfield	110 ± 3	80 ± 4	95	74 ± 5	67 ± 8	70	1.49 ± 0.12	1.21 ± 0.15	1.35	282 ± 12	189 ± 22	236
WB881	151 ± 6	85 ± 4	118	69 ± 8	89 ± 4	79	2.22 ± 0.32	0.96 ± 0.09	1.59	392 ± 32	252 ± 8	322
Commander	168 ± 4	126 ± 4	147	36 ± 2	53 ± 6	45	4.61 ± 0.20	2.39 ± 0.36	3.50	263 ± 15	263 ± 17	263
AC Navigator	138 ± 4	93 ± 2	116	40 ± 8	66 ± 5	53	3.60 ± 0.73	1.42 ± 0.10	2.51	222 ± 39	205 ± 12	214
DT724	157 ± 2	117 ± 4	137	51 ± 9	60 ± 6	56	3.15 ± 0.57	1.96 ± 0.22	2.56	311 ± 41	260 ± 16	286
Stewart-63	54 ± 1	34 ± 17	44	36 ± 7	27 ± 7	31	1.56 ± 0.32	1.21 ± 0.59	1.38	62 ± 10	27 ± 15	44
Arcola	76 ± 4	52 ± 2	64	115 ± 16	99 ± 9	107	0.67 ± 0.13	0.53 ± 0.05	0.60	237 ± 16	138 ± 8	188
'97EMMER19' AND EMMER-DERIVED BREEDING LINES												
97Emmer19	43 ± 6	34 ± 3	39	97 ± 16	73 ± 25	85	0.45 ± 0.08	0.52 ± 0.19	0.48	114 ± 26	75 ± 12	95
2000EB4	116 ± 9	78 ± 4	97	109 ± 18	113 ± 35	111	1.10 ± 0.25	0.78 ± 0.36	0.94	459 ± 52	305 ± 77	382
X.98.142.17	113 ± 8	88 ± 2	101	89 ± 11	90 ± 12	90	1.29 ± 0.20	0.99 ± 0.13	1.14	324 ± 43	259 ± 25	292
X.98.142.18	145 ± 3	-	-	58 ± 5	-	-	2.50 ± 0.19	-	-	329 ± 25	-	-
P.01.64.31	144 ± 2	94 ± 4	119	54 ± 5	80 ± 8	67	2.66 ± 0.25	1.18 ± 0.15	1.92	308 ± 17	262 ± 17	285
P.01.64.39	159 ± 5	106 ± 2	133	53 ± 4	65 ± 6	59	3.04 ± 0.26	1.64 ± 0.15	2.34	323 ± 18	254 ± 13	289
P.01.64.62	155 ± 9	95 ± 3	125	47 ± 10	73 ± 6	60	3.45 ± 0.81	1.30 ± 0.14	2.38	298 ± 54	245 ± 8	272
05Emmerreg-01	-	61 ± 2	-	-	71 ± 13	-	-	0.88 ± 0.15	-	-	142 ± 15	-
05Emmerreg-03	-	79 ± 4	-	-	53 ± 13	-	-	1.59 ± 0.48	-	-	150 ± 17	-
05Emmerreg-10	-	90 ± 5	-	-	100 ± 10	-	-	0.92 ± 0.13	-	-	287 ± 24	-
05Emmerreg-26	-	70 ± 4	-	-	95 ± 21	-	-	0.76 ± 0.18	-	-	218 ± 19	-
LSD ^h _{0.05}			31			34			1.71			100

Values represent mean ± standard deviation of five dough pieces per composite sample ^aP = Alveograph tenacity (1.1 x height) (mm) ^bL = Alveograph extensibility (mm) ^cP/L = Alveograph curve configuration ratio ^dW = Alveograph strength (J x 10⁻⁴) ^e 2005 = 2005 growing season ^f 2006 = 2006 growing season ^gC = Combined composite samples data across years (2005 and 2006) ^hLSD = Least Significant Difference

than the durum wheat checks ‘Commander’ and ‘Strongfield’ ($P < 0.05$) (Table 11). Of the emmer-derived breeding lines, ‘P.01.64.39’ and ‘P.01.64.62’ had P values similar to ‘Commander’ (Table 11). All of the USDA-ARS translocation lines had high P values that were statistically higher ($P < 0.05$) than ‘Strongfield’ (Table 11). As expected, ‘Commander’ (an extra-strong gluten durum; Clarke et al., 2006a) showed a higher P than ‘Strongfield’.

Although the dough extensibility of ‘97Emmer19’ was similar to ‘Strongfield’, its progeny ‘2000EB4’ had a significantly higher ($P < 0.05$) alveograph L values. All of the emmer-derived breeding lines tested in 2006 had similar L values to ‘Strongfield’, with the exception of ‘05Emmereg-10’ and ‘2000EB4’ which had L values similar to ‘AC Barrie’ and ‘AC Superb’ (Table 11). Doughs from ‘Strongfield’ and ‘WB881’ were more extensible ($P < 0.05$) than that of ‘Commander’, but no significant differences in L were detected between ‘Commander’, ‘AC Navigator’, ‘DT724’ and ‘Stewart-63’ (Table 11). The L value of ‘Arcola’ was 107 mm, significantly higher ($P < 0.05$) than all other durum wheat varieties evaluated (Table 11). Alveograph extensibilities (L) were substantially higher in bread wheat checks relative to the durum wheat varieties evaluated.

The genotype ‘2000EB4’ displayed a statistically lower P/L ($P < 0.05$) compared to USDA-ARS translocation lines, due to low P values and extensible dough, much like the bread wheat checks (Table 11). The P/L ratios for the remaining emmer-derived breeding lines that were evaluated over two composite samples were similar to ‘WB881’ and ‘AC Navigator’. The P/L ratio was high in the USDA-ARS translocation lines because of the high P and low dough extensibility observed in these lines, particularly ‘S99B33’ (Table 11). ‘2000EB4’ showed a numerically higher W value than did the bread wheat checks (Table 11). No other statistical differences were observed between durum wheat genotypes for alveograph W , including the emmer-derived breeding lines. Alveograph W values were lowest for ‘Stewart-63’, but no statistical difference was detected relative to ‘97Emmer19’ (Table 11).

4.3.3 Canadian Short Process Baking Results

For bake tests, the Canadian Short Process (CSP) bake test was used as the short fermentation process is best suited for comparing durum lines variable in gluten strength (Sapirstein et al. 2007) and starch damage (Dexter et al., 1994 and Marchylo et al., 2001).

Table 12. CSP^a Baking parameters (mean \pm standard deviation) of bread wheat checks (AC Barrie, CDC Teal, and AC Superb) and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines), for 2005, 2006 and combined composite data.

Genotype	Peak Mixing Time (min)			Loaf Volume (cc)			Loaf Shape (0-5 scale)		
	2005	2006	C ^b	2005	2006	C	2005	2006	C
BREAD WHEAT CHECK CULTIVARS									
AC Barrie	7.1 \pm 0.7	6.2 \pm 0.4	6.7	913 \pm 48	844 \pm 59	876	3.0 \pm 0.1	3.0 \pm 0.2	3.0
CDC Teal	6.8 \pm 0.6	6.5 \pm 0.6	6.7	968 \pm 29	891 \pm 72	927	3.1 \pm 0.1	3.0 \pm 0.1	3.1
AC Superb	5.6 \pm 0.3	5.0 \pm 0.3	5.3	897 \pm 50	803 \pm 42	850	2.9 \pm 0.3	2.9 \pm 0.2	2.9
1AS.1AL-1DL TRANSLOCATION LINES									
04EDUYT-43	3.1 \pm 0.3	-	-	538 \pm 32	-	-	1.1 \pm 0.1	-	-
L092	6.4 \pm 1.4	4.4 \pm 0.9	5.4	423 \pm 59	468 \pm 47	447	1.4 \pm 0.5	1.0 \pm 0.0	1.2
L252	5.0 \pm 2.8	7.3 \pm 1.3	6.2	513 \pm 18	498 \pm 103	505	1.7 \pm 0.9	1.8 \pm 0.8	1.7
S99B33	3.8 \pm 1.0	3.0 \pm 0.0	3.5	423 \pm 13	435 \pm 14	428	1.0 \pm 0.0	1.0 \pm 0.0	1.0
S99B34	2.7 \pm 0.5	2.7 \pm 0.1	2.8	437 \pm 55	463 \pm 39	445	1.0 \pm 0.0	1.0 \pm 0.0	1.0
DURUM WHEAT CULTIVARS									
Strongfield	5.9 \pm 0.4	5.3 \pm 0.3	5.6	725 \pm 26	723 \pm 31	724	2.1 \pm 0.6	2.6 \pm 0.1	2.4
WB881	7.6 \pm 0.4	5.9 \pm 0.3	6.7	765 \pm 33	773 \pm 34	769	2.4 \pm 0.5	2.7 \pm 0.0	2.6
Commander	6.7 \pm 0.2	5.6 \pm 0.3	6.1	722 \pm 29	726 \pm 39	724	2.4 \pm 0.3	2.5 \pm 0.1	2.5
AC Navigator	5.6 \pm 0.6	4.6 \pm 0.4	5.1	742 \pm 40	746 \pm 37	744	2.4 \pm 0.4	2.6 \pm 0.1	2.5
DT724	6.8 \pm 0.3	5.7 \pm 0.3	6.2	757 \pm 45	749 \pm 38	753	2.6 \pm 0.2	2.5 \pm 0.1	2.5
Stewart-63	2.7 \pm 0.1	2.1 \pm 0.1	2.4	645 \pm 53	680 \pm 29	664	1.5 \pm 0.3	1.3 \pm 0.2	1.4
Arcola	5.8 \pm 1.4	4.0 \pm 0.1	4.9	763 \pm 23	728 \pm 25	744	2.3 \pm 0.7	2.5 \pm 0.1	2.4
'97EMMER19' AND EMMER-DERIVED BREEDING LINES									
97Emmer19	3.7 \pm 1.0	3.1 \pm 0.4	3.4	862 \pm 16	764 \pm 31	810	2.8 \pm 0.1	2.6 \pm 0.0	2.7
2000EB4	9.0 \pm 2.3	6.2 \pm 0.3	7.6	793 \pm 6	785 \pm 28	789	2.4 \pm 0.6	2.7 \pm 0.2	2.5
X.98.142.17	7.3 \pm 1.1	6.0 \pm 0.3	6.6	783 \pm 53	810 \pm 44	798	2.4 \pm 0.7	2.8 \pm 0.1	2.6
X.98.142.18	8.3 \pm 0.5	-	-	772 \pm 31	-	-	2.6 \pm 0.2	-	-
P.01.64.31	7.0 \pm 1.1	5.9 \pm 0.6	6.4	812 \pm 32	773 \pm 35	791	2.6 \pm 0.1	2.8 \pm 0.1	2.7
P.01.64.39	6.4 \pm 1.1	5.5 \pm 0.1	5.9	778 \pm 24	783 \pm 33	781	2.3 \pm 0.7	2.7 \pm 0.1	2.5
P.01.64.62	9.8 \pm 1.7	7.1 \pm 0.4	8.4	767 \pm 8	754 \pm 40	760	2.5 \pm 0.6	2.7 \pm 0.1	2.6
05Emmerg-01	-	4.3 \pm 0.1	-	-	780 \pm 26	-	-	2.8 \pm 0.1	-
05Emmerg-03	-	4.5 \pm 0.5	-	-	815 \pm 53	-	-	2.7 \pm 0.2	-
05Emmerg-10	-	5.3 \pm 0.3	-	-	738 \pm 38	-	-	2.7 \pm 0.1	-
05Emmerg-26	-	4.6 \pm 0.3	-	-	800 \pm 40	-	-	2.7 \pm 0.2	-
LSD ^c _{0.05}			2.0			63			0.4

^aCSP = Canadian Short Process ^bC = Combined composite samples data across years (2005 and 2006)

^cLSD = Least significant difference

For the genotypes evaluated in both 2005 and 2006, the ANOVA for peak mixing time (min), loaf volumes (cc), loaf shape, crumb colour, and crumb structure are presented in Appendix A7.

4.3.3.1 Peak Mixing Time (PMT)

For bake tests, composite samples were mixed in a GRL mixer to “fully develop” the dough. Peak Mixing Time (PMT, min) was variable between lines, ranging from as low as 2.4 minutes for the weak gluten genotype ‘Stewart-63’ to 8.4 minutes for ‘P.01.64.62’. The PMT of ‘97Emmer19’ was not statistically different from ‘Stewart 63’, but lower than ‘Strongfield’, ‘WB881’ and ‘Commander’. The PMT of ‘2000EB4’ was twice as long as that of ‘97Emmer19’, one of its parents. Averaged over both years composite samples, ‘P.01.64.62’ displayed statistically higher ($P<0.05$) PMT than the current durum varieties ‘Strongfield’, ‘Commander’ and ‘AC Navigator’ (Table 12). The USDA-ARS translocation lines were statistically similar to ‘Strongfield’, ‘Commander’ and ‘AC Navigator’, but large variation in PMT was evident between yearly composite samples. The standard deviation (SD) of triplicate mixings of USDA-ARS translocation lines was considerably higher ($P<0.05$) than the SD of samples from other genotypes evaluated, especially in 2005 (Table 12). In contrast, the PMT of ‘S99B33’ and ‘S99B34’ in the 2006 composite samples were lower ($P<0.05$) than most durum wheat genotypes evaluated (Table 12). The PMTs of bread wheat checks were statistically similar to the durum wheat genotypes ($P>0.05$), with the exception of ‘Stewart-63’ (Table 12).

4.3.3.2 Loaf Volume (LV)

Averaged over yearly composite samples, the LV of ‘97Emmer19’ was 810 cc, which was not statistically different from the bread wheat check ‘AC Superb’ and was statistically higher ($P<0.05$) than all durum wheat checks with the exception of ‘WB881’ and ‘DT724’ (Table 12). The emmer-derived breeding lines ‘2000EB4’, ‘X98.142.17’, ‘P01.64.31’ and ‘P01.64.39’ had LV 20-30 cc greater ($P<0.05$) than ‘WB881’ and significantly greater ($P<0.05$) LV than ‘Strongfield’ and ‘Commander’. In 2006, ‘X.98.142.17’, ‘05Emmereg-03’ and ‘05Emmereg-26’ had LV similar to ‘AC Superb’ (Table 12). These results confirm those of Schlichting et al. (2002) who reported that emmer-derived breeding lines produced bread with superior LV than the durum wheat check ‘WB881’. Despite carrying the 1AS.1Al-1DL translocation, the LV of the USDA-

Table 13. CSP^a Baking parameters (mean ± standard deviation) of bread wheat checks, AC Barrie, CDC Teal, and AC Superb, and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation, and emmer-derived breeding lines), for 2005, 2006 and combined composite data.

Genotype	Crumb Structure (0-5 scale)			Crumb Colour (0-5 scale)		
	2005	2006	C ^b	2005	2006	C
BREAD WHEAT CHECK CULTIVARS						
AC Barrie	3.0 ± 0.1	3.0 ± 0.2	3.0	2.9 ± 0.1	3.0 ± 0.1	2.9
CDC Teal	3.1 ± 0.1	3.0 ± 0.1	3.0	3.0 ± 0.0	3.0 ± 0.0	3.0
AC Superb	3.0 ± 0.2	3.0 ± 0.1	3.0	3.0 ± 0.1	3.0 ± 0.1	3.0
1AS.1AL-1DL TRANSLOCATION LINES						
04EDUYT-43	1.0 ± 0.0	-	-	1.0 ± 0.0	-	-
L092	0.8 ± 0.3	1.1 ± 0.1	1.0	1.1 ± 0.1	1.3 ± 0.7	1.2
L252	1.4 ± 1.2	1.6 ± 0.6	1.5	1.3 ± 0.4	2.5 ± 0.1	1.9
S99B33	1.1 ± 0.7	1.0 ± 0.0	1.1	1.1 ± 0.2	1.0 ± 0.0	1.0
S99B34	1.2 ± 0.9	1.1 ± 0.1	1.2	1.2 ± 0.3	1.4 ± 0.8	1.3
DURUM WHEAT CULTIVARS						
Strongfield	2.4 ± 0.1	2.5 ± 0.1	2.4	1.5 ± 0.0	2.3 ± 0.4	1.9
WB881	2.4 ± 0.1	2.6 ± 0.1	2.5	1.3 ± 0.2	2.2 ± 0.4	1.8
Commander	2.5 ± 0.0	2.5 ± 0.0	2.5	1.1 ± 0.2	1.5 ± 0.4	1.3
AC Navigator	2.5 ± 0.0	2.5 ± 0.0	2.5	1.7 ± 0.5	2.1 ± 0.4	1.9
DT724	2.6 ± 0.1	2.5 ± 0.1	2.5	1.6 ± 0.2	2.3 ± 0.4	2.0
Stewart-63	1.8 ± 0.6	1.3 ± 0.1	1.5	1.8 ± 0.5	2.6 ± 0.1	2.2
Arcola	2.5 ± 0.1	2.4 ± 0.1	2.4	1.5 ± 0.0	2.5 ± 0.0	2.0
'97EMMER19' AND EMMER-DERIVED BREEDING LINES						
97Emmer19	2.3 ± 0.1	2.4 ± 0.1	2.3	2.5 ± 0.1	2.7 ± 0.1	2.6
2000EB4	2.6 ± 0.1	2.5 ± 0.2	2.6	2.2 ± 0.5	2.6 ± 0.1	2.4
X.98.142.17	2.5 ± 0.2	2.7 ± 0.0	2.6	1.5 ± 0.0	2.3 ± 0.3	1.9
X.98.142.18	2.6 ± 0.1	-	-	2.0 ± 0.0	-	-
P.01.64.31	2.6 ± 0.0	2.5 ± 0.1	2.6	1.5 ± 0.1	2.3 ± 0.4	1.9
P.01.64.39	2.5 ± 0.2	2.7 ± 0.1	2.6	1.5 ± 0.0	2.1 ± 0.4	1.8
P.01.64.62	2.5 ± 0.2	2.5 ± 0.1	2.5	1.4 ± 0.2	2.1 ± 0.4	1.8
05Emmereg-01	-	2.5 ± 0.2	-	-	2.1 ± 0.4	-
05Emmereg-03	-	2.8 ± 0.1	-	-	2.3 ± 0.5	-
05Emmereg-10	-	2.4 ± 0.1	-	-	2.2 ± 0.6	-
05Emmereg-26	-	2.6 ± 0.2	-	-	2.2 ± 0.5	-
LSD ^c _{0.05}			0.3			0.5

^aCSP = Canadian Short Process

^bC = Combined data over two years (2005 and 2006)

^cLSD = Least significant difference

ARS translocation lines were nearly 50% lower than the bread wheat checks, and statistically lower ($P < 0.05$) than all durum wheat varieties evaluated, including ‘Stewart-63’.

Of the durum wheat genotypes, ‘Stewart-63’ had the lowest LV (664 cc) of the durum wheat genotypes averaging over yearly composite samples (Table 12). Of the durum wheat check cultivars, ‘WB881’ had the highest LV of the durum wheat checks, averaging 769 cc, but no statistical differences were noted between ‘WB881’, ‘Strongfield’, ‘Commander’, ‘AC Navigator’, ‘Arcola’ and ‘DT724’. Averaged over composite samples, the LV of bread wheat checks ranged from 850 cc for ‘AC Superb’ to 927 cc for ‘CDC Teal’ (Table 12). ‘CDC Teal’ was included in the bake tests as it consistently displays superior baking performance in local baking tests and as a check cultivar in co-operative registration trials (P. Hucl, personal communication).

4.3.3.3 Loaf Shape (LS), Crumb Structure (CS), and Crumb Colour (CC)

All of the emmer-derived breeding lines (including the 05Emmereg series lines evaluated in 2006) and durum genotypes with the exception of ‘Strongfield’ and ‘Arcola’, showed no significant differences in LS compared to the bread wheat check ‘AC Superb’ (Table 12). Overall the USDA-ARS translocation lines exhibited unappealing loaf shape and crumb structure. In both years of testing, the USDA-ARS translocation lines exhibited very little oven rise and had uneven loaf shape with coarse side walls and had no break and shred (Table 13). ‘Stewart-63’ was not significantly different from USDA-ARS translocation lines in LS and CS scorings, although it showed significantly higher ($P < 0.05$) LV than USDA-ARS translocation lines (Tables 12 and 13).

As expected, the durum wheat checks had a lower CC scores than the bread wheat check cultivars (Table 13). In this case, the lower CC score of durum wheat checks and some emmer-derived breeding lines does not mean “of poorer quality”, but indicating a “more yellow” appearance of bread crumb. ‘Commander’, which had the highest semolina b^* (Table 7), had the lowest CC score (Table 13). The correlation between LS means of CC and flour/semolina yellowness (b^*) (see results section 4.2.2; Table 7) was -0.79 ($P < 0.001$).

4.4 Correlation Coefficients between Bread-making Quality-related Characteristics

Given the genetic differences in LV potential observed among durum wheat check

Table 14. Correlation Coefficients (from combined least square means of only tetraploid genotypes grown in both the 2005 and 2006 growing seasons) between bread-making quality-related characteristics.

	HI ^a	SDS ^b	GPC ^c	FN ^d	GI ^e	WG ^f	DG ^g	F/SP ^h	FAB ⁱ	DDT ^j	MTI ^k	TTB ^l	STA ^m	P ⁿ	L ^o	P/L ^p	W ^q	LV ^r	
SDS	ns																		
GPC	ns	ns																	
FN	ns	ns	ns																
GI	ns	.81**	ns	ns															
WG	-.68**	ns	ns	ns	ns														
DG	-.72**	ns	ns	ns	ns	.93**													
F/SP	ns	ns	.98**	ns	ns	ns	ns												
FAB	.71**	ns	ns	ns	ns	ns	ns	ns											
DDT	ns	ns	ns	ns	.57*	-.71**	-.56*	ns	ns										
MTI	-.58*	-.67**	ns	ns	-.93**	.57*	ns	ns	ns	-.74**									
TTB	.49*	ns	ns	ns	.56*	-.74**	-.58*	ns	ns	.98**	-.74**								
STA	.48*	ns	ns	ns	.60**	-.70**	-.54*	.47*	ns	.96**	-.79**	.97**							
P	.77**	ns	ns	ns	.81**	-.56*	ns	ns	.74**	.59*	-.87**	.60**	.61**						
L	-.67**	ns	ns	.63**	ns	.61**	.70**	ns	-.63**	ns	ns	ns	ns	-.47*					
P/L	.77**	ns	ns	ns	.51*	-.74**	-.66**	ns	.69**	.73**	-.69**	.75**	.73**	.85**	-.77**				
W	ns	.71**	ns	ns	.88**	ns	ns	ns	ns	ns	-.79**	ns	ns	.69**	ns	ns			
LV	-.51*	ns	ns	.57*	ns	.74**	.64**	ns	ns	-.89**	.53*	-.90**	-.86**	-.51*	.62**	-.78**	ns		
PMT ^s	ns	.49*	ns	.53*	.54*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	.70**	ns	

* $P < 0.05$ ** $P < 0.01$ ns non significant at $P = 0.05$

^aHI = Hardness Index ^bSDS = SDS sedimentation volumes (cc) ^cGPC = Grain Protein Concentration (13.5% moisture basis)

^dFN = Falling Number (sec) ^eGI = semolina Gluten Index (%) ^fWG = semolina Wet Gluten (%)

^gDG = semolina Dry Gluten (%) ^hF/SP = flour protein/semolina protein concentration (14.0% moisture basis)

ⁱFAB = Farinograph Water Absorption (%) ^jDDT = Farinograph Dough Development Time (min)

^kMTI = Farinograph Mixing Tolerance Index (B.U) ^lTTB = Farinograph Time To Breakdown (min)

^mSTA = Farinograph Stability (min) ⁿP = Alveograph tenacity (1.1xheight) (mm) ^oL = Alveograph extensibility (mm)

^pP/L = Alveograph curve configuration ratio ^qW = Alveograph baking strength ($J \times 10^{-4}$) ^rLV = Loaf Volume (cc)

^sPMT = Peak Mixing Time

Table 15. Correlation Coefficients (from combined least square means of tetraploid genotypes excluding USDA-ARS translocation lines grown in both the 2005 and 2006 growing seasons) between bread-making quality-related characteristics.

	HI ^a	SDS ^b	GPC ^c	FN ^d	GI ^e	WG ^f	DG ^g	F/SP ^h	FAB ⁱ	DDT ^j	MTI ^k	TTB ^l	STA ^m	P ⁿ	L ^o	P/L ^p	W ^q	LV ^r	
SDS	ns																		
GPC	ns	ns																	
FN	ns	ns	ns																
GI	ns	.85**	ns	.55*															
WG	-.57*	ns	.88**	ns	ns														
DG	-.64*	ns	.78**	ns	ns	.92**													
F/SP	ns	ns	.99**	ns	ns	.88**	.81**												
FAB	.71**	ns	ns	ns	ns	ns	ns	ns											
DDT	ns	.80**	ns	.61*	.72**	ns	ns	ns	ns										
MTI	ns	-.74**	ns	ns	-.95**	ns	ns	ns	ns	-.71**									
TTB	ns	.76**	ns	ns	.73**	ns	ns	ns	ns	.97**	-.76**								
STA	ns	.71**	ns	.56*	.62*	ns	ns	ns	ns	.87**	-.70**	.92**							
P	.70**	ns	ns	ns	.76**	ns	ns	ns	.73**	ns	-.80**	ns	ns						
L	-.58*	.60*	ns	.55*	ns	ns	.59*	ns	-.63*	.57*	ns	ns	ns	ns					
P/L	.72**	ns	ns	ns	ns	ns	ns	ns	.84**	ns	ns	ns	ns	.83**	-.71**				
W	ns	.79**	ns	.65*	.93**	ns	ns	ns	ns	.76**	-.94**	.80**	.71**	.75**	ns	ns			
LV	ns	.82**	ns	ns	.62*	ns	.55*	ns	ns	.57*	ns	ns	ns	ns	.62*	ns	ns		
PMT ^s	ns	.76**	ns	ns	.92**	ns	ns	ns	ns	.75**	-.95**	.82**	.79**	.72**	ns	ns	ns	.91**	ns

* $P < 0.05$ ** $P < 0.01$ ns non significant at $P = 0.05$

^aHI = Hardness Index ^bSDS = SDS sedimentation volumes (cc) ^cGPC = Grain Protein Concentration (13.5% moisture basis)

^dFN = Falling Number (sec) ^eGI = semolina Gluten Index (%) ^fWG = semolina Wet Gluten (%)

^gDG = semolina Dry Gluten (%) ^hF/SP = flour protein/semolina protein concentration (14.0% moisture basis)

ⁱFAB = Farinograph Water Absorption (%) ^jDDT = Farinograph Dough Development Time (min)

^kMTI = Farinograph Mixing Tolerance Index (B.U) ^lTTB = Farinograph Time To Breakdown (min)

^mSTA = Farinograph Stability (min) ⁿP = Alveograph tenacity (1.1xheight) (mm) ^oL = Alveograph extensibility (mm)

^pP/L = Alveograph curve configuration ratio ^qW = Alveograph baking strength ($J \times 10^{-4}$) ^rLV = Loaf Volume (cc)

^sPMT = Peak Mixing Time

cultivars, '97Emmer19' and the emmer-derived breeding lines in two years of testing (Section 4.3.3), correlation coefficients were estimated to determine the chemical and rheological properties most associated with variation in LV (Table 14). Since the objective of this research was to assess rheological properties associated with enhanced baking in durum wheats, data from the bread wheat checks were excluded from the correlation analyses (Table 14). Because the USDA-ARS 1AS.1AL-1DL translocation lines exhibited distinct rheological properties (very strong, inextensible gluten) compared to other durum wheat lines, correlations were also assessed with these data removed (Table 15).

Significant correlations were observed among the farinograph and alveograph parameters measured in this study, with the exception of alveograph *L*, which only correlated with FAB (Table 14). Alveograph *W* and tenacity (*P*, an indicator of dough elasticity) were strongly correlated ($P < 0.01$) to GI ($r = 0.88$; $P < 0.01$ and 0.81 ; $P < 0.01$, respectively) (Table 14), which are in agreement with the study by Sapirstein et al. (2007). A significant, positive correlation was seen between GI and SDS sedimentation volumes and is in agreement with the studies by Dexter et al. (1981) and Dessalegn et al. (2006) (Table 14). Positive correlations between GI and alveograph *P* and *W* were observed (Table 14), which are in agreement with the study by Edwards et al. (2007).

Variation in LV of all durum wheat genotypes, including the USDA-ARS translocation lines, was positively correlated with semolina wet gluten and dry gluten content ($r = 0.74$; $P < 0.01$; $r = 0.64$; $P < 0.01$, respectively) and falling number ($r = 0.57$; $P < 0.05$) (Table 14). A significant positive correlation was also observed between LV and alveograph extensibility *L* ($r = 0.62$; $P < 0.01$) and farinograph mixing tolerance index (MTI; $r = 0.53$; $P < 0.05$). In contrast, significant ($P < 0.05$) negative correlations with LV were observed for farinograph time to breakdown (TTB; $r = -0.90$; $P < 0.01$), farinograph dough development time DDT ($r = -0.89$; $P < 0.01$), farinograph stability STA ($r = -0.86$; $P < 0.01$), alveograph curve configuration ratio *P/L* ($r = -0.78$; $P < 0.01$), alveograph tenacity *P* ($r = -0.51$; $P < 0.05$) and grain hardness ($r = -0.51$; $P < 0.05$) (Table 14). However, when the data from the USDA-ARS translocation lines were removed, only SDS ($r = 0.82$; $P < 0.01$), GI (0.62 ; $P < 0.05$), DDT (0.57 ; $P < 0.05$) and alveograph extensibility *L* (0.62 ; $P < 0.05$) were significantly ($P < 0.05$) associated with LV (Table 15),

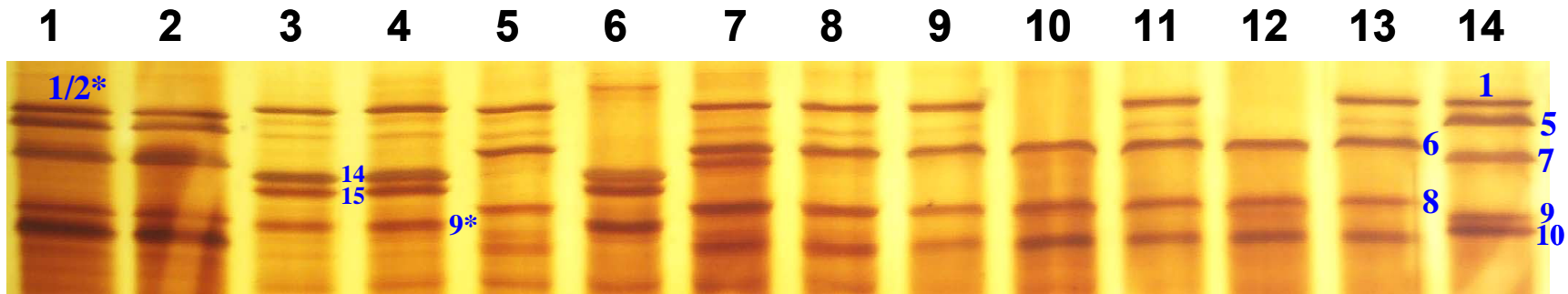
suggesting that both increased dough extensibility (*L*) and increased gluten strength are important to improved LV in durum wheats. No significant correlation was observed between LV and GPC (Table 14 and Table 15). However, loaf volumes showed significant correlation ($r = 0.55$; $P < 0.05$) with the dry gluten content (Table 15).

4.5 Electrophoresis Results

The high molecular weight glutenin subunits (*Glu-A1*, *Glu-B1*, and *Glu-D1* alleles) and gliadins (*Gli* loci) were identified based on SDS (Figures 2.1, 2.2, and 2.3) and Acid-PAGE gels (Figure 3.1 and 3.2), respectively. All genotypes evaluated in 2005 and 2006 field trials were included in the analysis. In addition, the bread wheat checks ‘Neepawa’, ‘Chinook’ and ‘Marquis’ have been well characterized for their subunit composition (Payne and Lawrence, 1983; Ng et al., 1988b) and were included to aid in identification of HMW-GS in ‘97Emmer19’ and the emmer-derived breeding lines. The allelic compositions of each of the genotypes evaluated are summarized in Table 16.

The HMW-GS ‘1Ax1’ (at *Glu-A1* locus) was identified in ‘97Emmer19’ and the emmer-derived breeding lines ‘2000EB4’, X.98.142.17’, ‘P.01.64.31’, ‘P.01.64.62’, ‘05Emmereg-01’, ‘05Emmereg-10’ and ‘05Emmereg-26’ (Figure 2.1 and Table 16). At *Glu-B1* locus, ‘97Emmer19’, ‘2000EB4’, and ‘05Emmereg-03’ carried the ‘Bx14+By15’ allelic combination with 9* protein subunit (Figure 2.1 and Table 16). The remaining emmer-derived breeding lines carried ‘Bx6+By8’ (Figure 2.1 and Table 16). At *Glu-B1* locus, ‘05Emmereg-10’ was heterogenous carrying both *Glu-B1a* (Bx7) and *Glu-B1d* (Bx6+By8) alleles (Figure 2.1 and Table 16).

Similar to previous results (Klindworth et al., 2005), the four 1AS.1AL-1DL translocation lines ‘L092’, ‘L252’, ‘S99B33’ and ‘S99B34’, had the null allele at the *Glu-A1* locus and Bx6+By8 allele at *Glu-B1* locus, but carried the translocated Dx5+Dy10 alleles from the D genome at *Glu-D1* locus (Figure 2.2). At the beginning of this study, the CIMMYT lines evaluated were believed to carry the 1AS.1AL-1DL translocation carrying the Dx5+Dy10 alleles at the *Glu-D1* locus, but electrophoresis confirmed that these lines lacked the translocation (Figure 2.3 and Table 16). As such, the CIMMYT lines were excluded from 2006 testing. CIMMYT translocation lines had a null allele at *Glu-A1* locus, Bx7+By8 allele at *Glu-B1* locus (Figure 2.3 and Table 16). All of the durum genotypes included in this study carried the null allele at *Glu-A1* locus



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Figure 2.1. SDS-PAGE (Sodium dodecyl sulphate – Polyacrylamide gel electrophoresis) profiles of 97Emmer19, 2000EB4, and emmer derived breeding lines – localization of HMW-GS. The bread wheat cultivars Chinook (Lane 1 and 2) and Marquis (Lane 14) are used as check cultivars to determine the specific molecular weights of HMW-GS seen in emmer accessions.

Lane 01 and 02 – Chinook; Lane 03 – 97Emmer19; Lane 04 – 2000EB4; Lane 05 – 05Emmereg-01; Lane 06 – 05Emmereg-03; Lane 07 – 05Emmereg-10; Lane 08 – 05Emmereg-26; Lane 09 – X.98.142.17; Lane 10 – X.98.142.18; Lane 11 – P.01.64.31; Lane 12 – P.01.64.39; Lane 13 – P.01.64.62; Lane 14 – Marquis

Molecular weight of protein subunits in KDa (Refer Appendix C1):

Subunit 1 = 149 KDa; 1/2* = 141; 5 = 128; 6 = 121; 7 = 115; 14 = 112; 15 = 107; 8 = 101; 9 = 95.7; 10 = 92.1

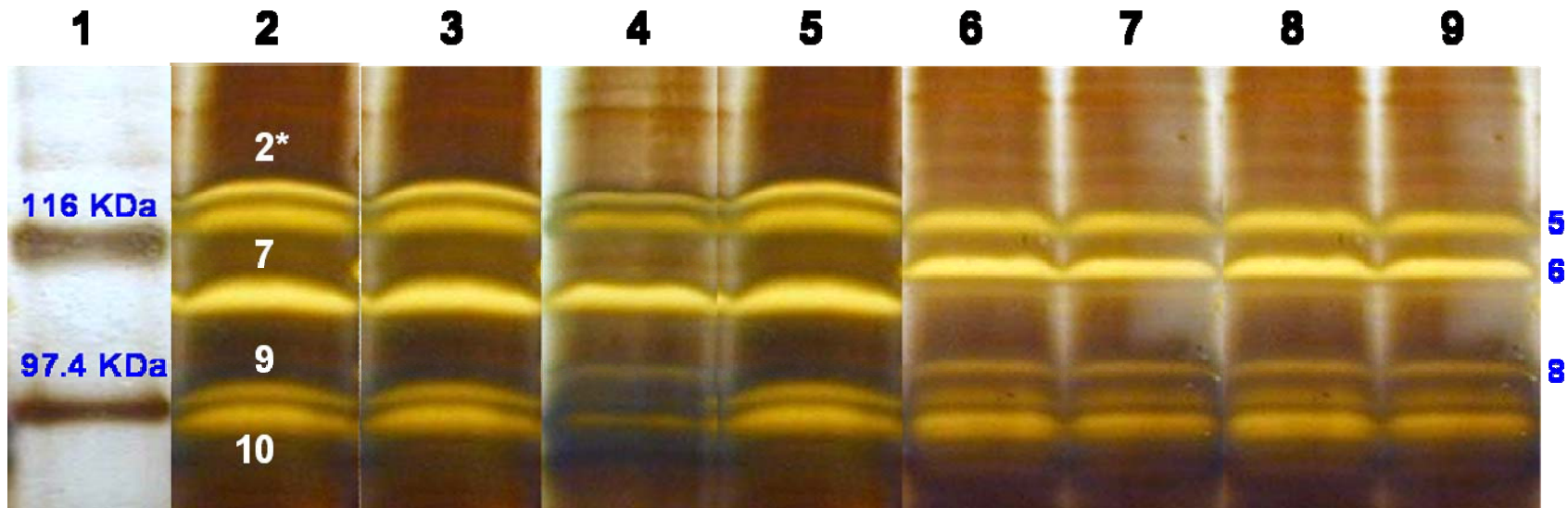


Figure 2.2. SDS-PAGE (Sodium dodecyl sulphate – Polyacrylamide gel electrophoresis) profiles of bread wheat checks and USDA translocation lines – localization of HMW-GS. The bread wheat cultivar Neepawa (Lane 1) and HMW-STD (Lane 2) are used as controls to determine the specific molecular weights of HMW-GS.

Lane 01 – HMW standard; Lane 02 – Neepawa; Lane 03 – AC Barrie; Lane 04 – CDC Teal; Lane 05 – AC Superb; Lane 06 – L092; Lane 07 – L252; Lane 08 – S99B33; Lane 09 – S99B34

Molecular weight of protein subunits in KDa (Appendix C1):

Subunit 2* = 136 KDa; 5 = 128; 6 = 121; 7 = 115; 8 = 101; 9 = 95.7; 10 = 92.1

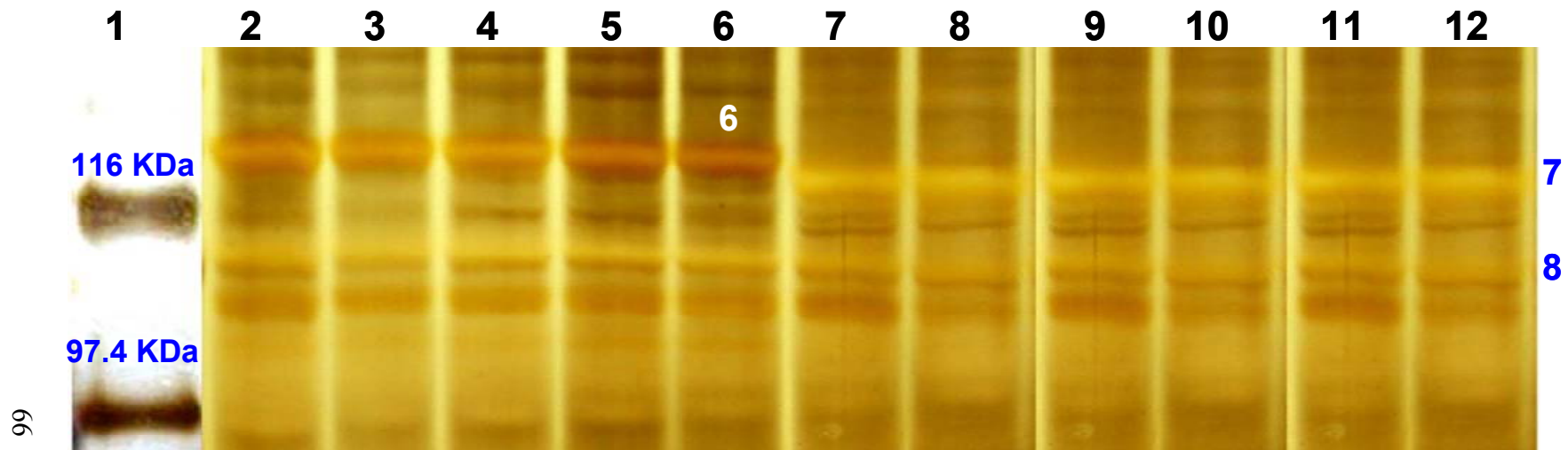


Figure 2.3. SDS-PAGE (Sodium dodecyl sulphate – Polyacrylamide gel electrophoresis) profiles of durum genotypes and CIMMYT translocation lines – localization of HMW-GS. The HMW-STD (Lane 1) was used as standard marker to determine the specific molecular weights of HMW-GS.

Lane 01 – HMW standard; Lane 02 – Strongfield; Lane 03 – WB881; Lane 04 – Commander; Lane 05 – AC Navigator; Lane 06 – DT 724; Lane 07 – Stewart-63; Lane 08 – Arcola; Lane 09 – 04EDUYT-42; Lane 10 – 04EDUYT-43; Lane 11 – 04IDSN-107; Lane 12 – 04IDSN-111

Molecular weight of protein subunits in KDa (Refer Appendix C1):

Subunit 6 = 121 KDa; 7 = 115; 8 = 101

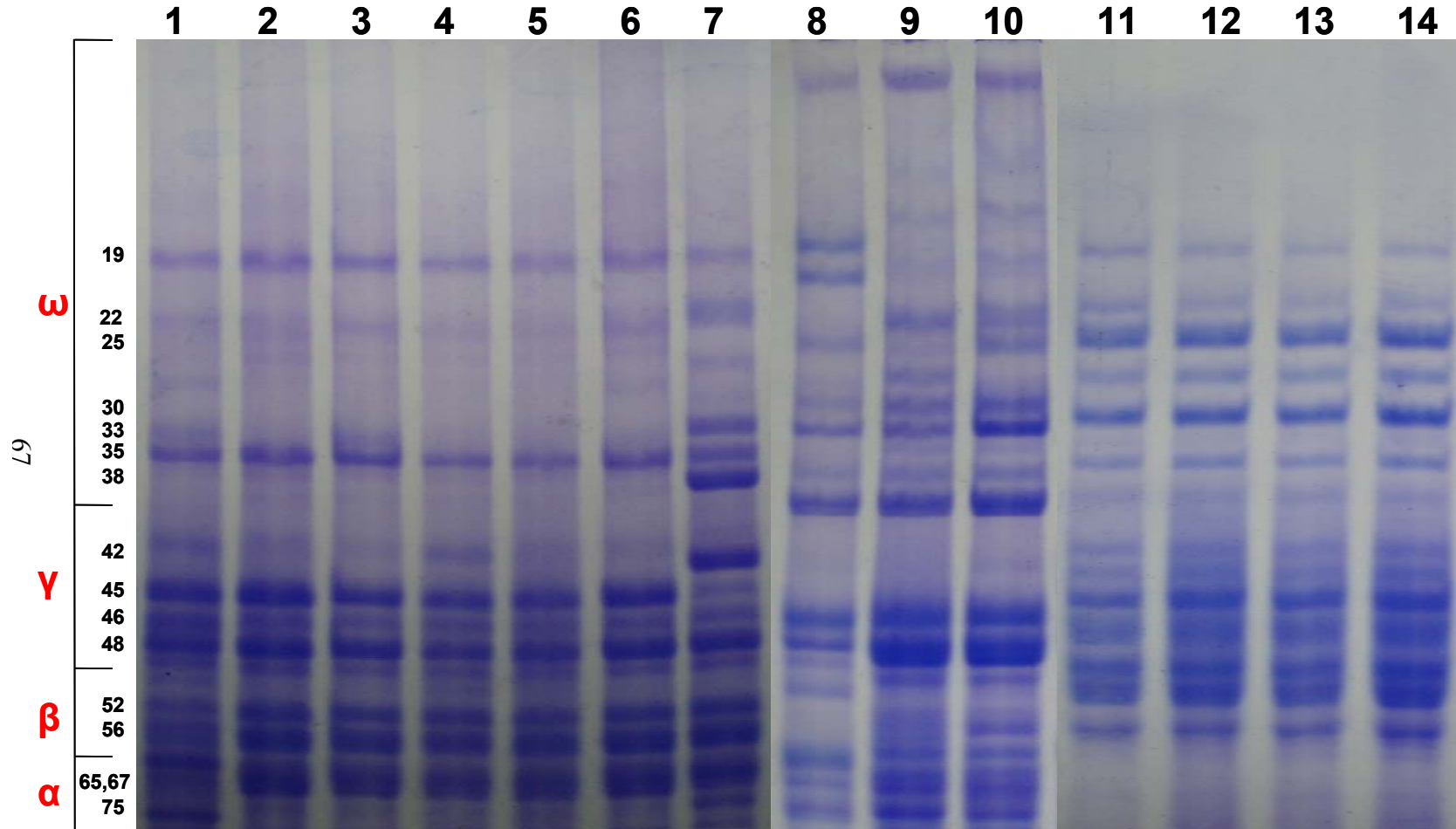


Figure 3.1. A-PAGE (Acid – Polyacrylamide gel electrophoresis) profiles of wheat gliadins: localization of 42- γ and 45- γ gliadins. The numbers on the left are the relative mobility (RM) values of the genotypes, calculated based on Lane 7 (Stewart-63).

Lane 01 – Strongfield; Lane 02 – WB881; Lane 03 – Commander; Lane 04 – AC Navigator; Lane 05 – DT724; Lane 06 – Arcola; Lane 07 – Stewart-63; Lane 08 – AC Superb; Lane 09 – CDC Teal; Lane 10 – AC Barrie; Lane 11 – 04EDUYT-42; Lane 12 – 04EDUYT-43; Lane 13 – 04IDSN-107; Lane 14 – 04IDSN-111

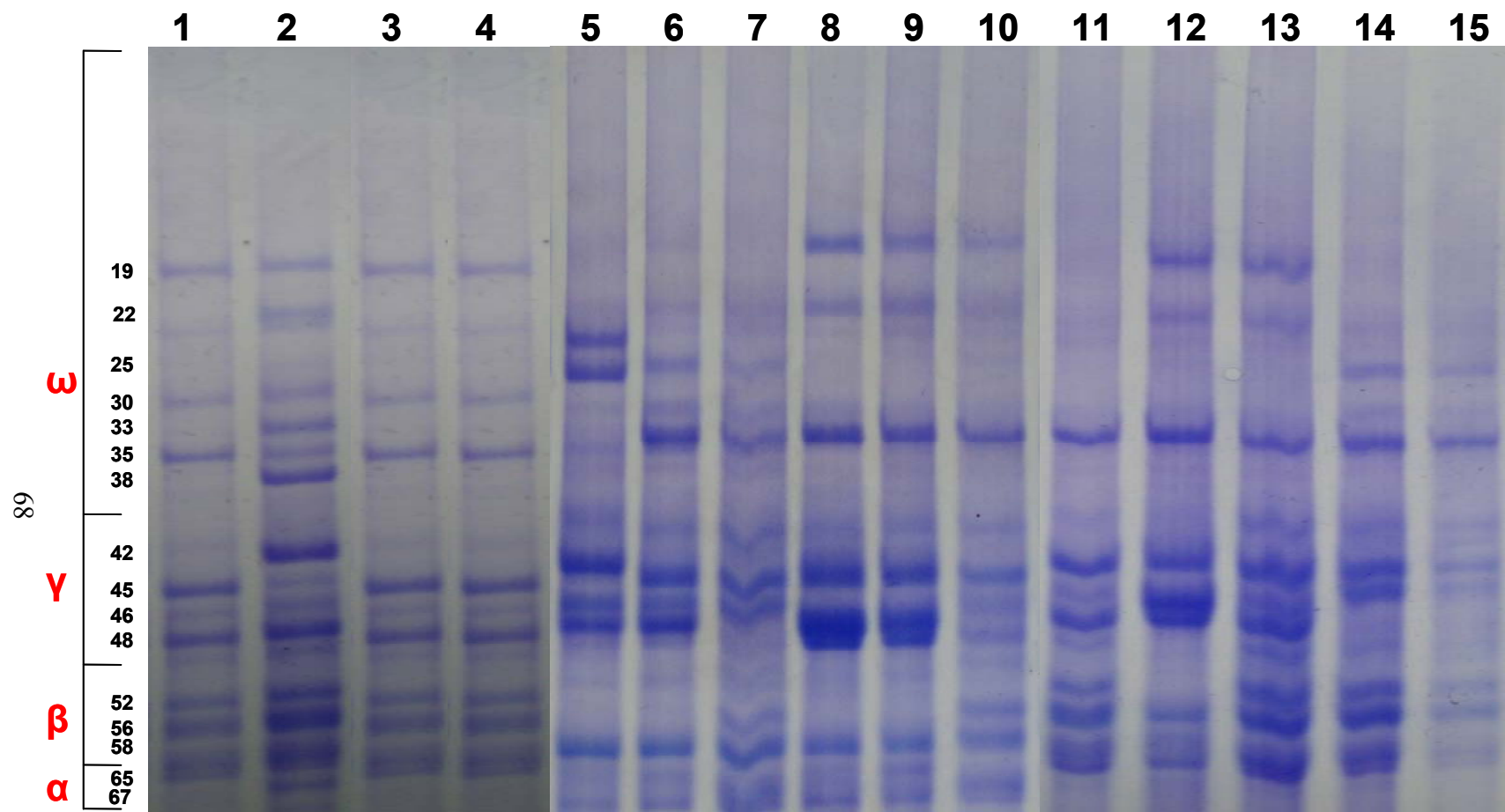


Figure 3.2. A-PAGE (Acid – Polyacrylamide gel electrophoresis) profiles of wheat gliadins: localization of 42- γ and 45- γ gliadins. The numbers on the left are the relative mobility (RM) values of the genotypes, calculated based on Lane 2 (L252).

Lane 01 – L092; Lane 02 – L252; Lane 03 – S99B33; Lane 04 – S99B34; Lane 05 – 97Emmer19; Lane 06 – 2000EB4; Lane 07 – 05Emmereg-01; Lane 08 – 05Emmereg-03; Lane 09 – 05Emmereg-10; Lane 10 – 05Emmereg-26; Lane 11 – X.98.142.17; Lane 12 – X.98.142.18; Lane 13 – P.01.64.31; Lane 14 – P.01.64.39; Lane 15 – P.01.64.62

Table 16. Summary of HMW-GS^a and Gliadin protein subunits detected in bread wheat checks (AC Barrie, CDC Teal, AC Superb, Neepawa, Chinook, and Marquis), and tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation and emmer-derived breeding lines).

Genotype	HMWGS			Gliadins				LMW ^b
	<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>	ω^f	γ^c	β^d	α^e	
BREAD WHEAT CHECK CULTIVARS								
Neepawa	<i>1Ax2*</i>	<i>Bx7+By9</i>	<i>Dx5+Dy10</i>					
Chinook	<i>1Ax1/2*</i>	<i>Bx7+By9</i>	<i>Dx5+Dy10</i>					
Marquis	<i>1Ax1</i>	<i>Bx7+By9</i>	<i>Dx5+Dy10</i>					
AC Barrie	<i>1Ax2*</i>	<i>Bx7+By9</i>	<i>Dx5+Dy10</i>	25,30	46,48	52,56	67,75	
CDC Teal	<i>1Ax2*</i>	<i>Bx7+By8</i>	<i>Dx5+Dy10</i>	25,30	46,48	52,56	67,75	
AC Superb	<i>1Ax2*</i>	<i>Bx7+By9</i>	<i>Dx5+Dy10</i>	19,30	46,48	56	67,75	
1AS.1AL-1DL TRANSLOCATION LINES								
04-EDUYT-42	Null	<i>Bx7+By8</i>		19,25,30	45,46	56		2
04-EDUYT-43	Null	<i>Bx7+By8</i>		19,25,30	45,46	56		2
04-IDSN-107	Null	<i>Bx7+By8</i>		19,25,30	45,46	56		2
04-IDSN-111	Null	<i>Bx7+By8</i>		19,25,30	45,46	56		2
L092	Null	<i>Bx6+By8</i>	<i>Dx5+Dy10</i>	35	45,48	52,56	65,67	2
L252	Null	<i>Bx6+By8</i>	<i>Dx5+Dy10</i>	19,22,33,35,38	42,48	52,56	65,67	1
S99B33	Null	<i>Bx6+By8</i>	<i>Dx5+Dy10</i>	35	45,48	52,56	65,67	2
S99B34	Null	<i>Bx6+By8</i>	<i>Dx5+Dy10</i>	35	45,48	52,56	65,67	2
DURUM WHEAT CULTIVARS								
Strongfield	Null	<i>Bx6+By8</i>		19,35	45,48	52,56	65,67	2
WB881	Null	<i>Bx6+By8</i>		19,35	45,48	52,56	65	2
Commander	Null	<i>Bx6+By8</i>		19,35	45,48	52,56	65	2
AC Navigator	Null	<i>Bx6+By8</i>		19,35	45,48	52,56	65	2
DT 724	Null	<i>Bx6+By8</i>		19,35	45,48	52,56	65	2
Stewart-63	Null	<i>Bx7+By8</i>		19,22,33,35,38	42,48	52,56	65,67	1
Arcola	Null	<i>Bx7+By8</i>		19,35	45,48	52,56	65	2
'97EMMER19' AND EMMER-DERIVED BREEDING LINES								
97Emmer19	<i>1Ax1</i>	<i>Bx14+By15;9*</i>		30,33	44,46,48	58		
2000EB4	<i>1Ax1</i>	<i>Bx14+By15;9*</i>		35	45,46,48	58		2
X.98.142.17	<i>1Ax1</i>	<i>Bx6+By8</i>		35	45,48	58		2
X.98.142.18	Null	<i>Bx6+By8</i>		35	45,48	58		2
P.01.64.31	<i>1Ax1</i>	<i>Bx6+By8</i>		35	45,48	58		2
P.01.64.39	Null	<i>Bx6+By8</i>		35	45,46	58		2
P.01.64.62	<i>1Ax1</i>	<i>Bx6+By8</i>		35	45,46	58		2
05Emmereg-01	<i>1Ax1</i>	<i>Bx6+By8</i>		35	45,46	58	65	2
05Emmereg-03	Null	<i>Bx14+By15;9*</i>		22,35	45,48	58	65	2
05Emmereg-10	<i>1Ax1</i>	<i>Bx6+By8;7</i>		22,35	45,48	58	65	2
05Emmereg-26	<i>1Ax1</i>	<i>Bx6+By8</i>		22,35	45,48	58	65	2

^aHMW-GS = High Molecular Weight Glutenin Subunit ^bLMW = Low Molecular Weight group ^c α -gliadins = alpha gliadins ^d β -gliadins = beta gliadins ^e γ -gliadins = gamma-gliadins ^f ω -gliadins = omega gliadins

and HMW-GS pair (alleles) *Bx6+By8* at *Glu-B1* locus, with the exception of ‘Stewart-63’ and ‘Arcola’ which carried *Bx7+By8* at the *Glu-B1* locus (Figure 2.3 and Table 16).

The bread wheat checks used for electrophoresis experiments included ‘Neepawa’ (*1Ax2** at *Glu-A1* locus, *Bx7+By9* at *Glu-B1* locus, and *Dx5+Dy10* at *Glu-D1* locus), ‘Chinook’ (*1Ax1/2** at *Glu-A1* locus, *Bx7+By9* at *Glu-B1* locus, and *Dx5+Dy10* at *Glu-D1* locus) and ‘Marquis’ (*1Ax1* at *Glu-A1* locus, *Bx7+By9* at *Glu-B1* locus, and *Dx5+Dy10* at *Glu-D1* locus) (Figures 2.1 and 2.2; Table 16). The *1Ax1/2** band (at *Glu-A1* locus) identified in ‘Chinook’ had electrophoretic mobility in between *1Ax1* and *1Ax2** subunits (Figure 2.1) (Ng et al., 1988b). Like ‘Neepawa’, the bread wheat checks ‘AC Superb’, ‘AC Barrie’ and ‘CDC Teal’ showed the *1Ax2** allele at the *Glu-A1* locus (Figure 2.2 and Table 16). ‘AC Superb’ and ‘AC Barrie’ carried *Bx7+By9* at *Glu-B1* locus and *Dx5+Dy10* alleles at the *Glu-D1* locus. In contrast, ‘CDC Teal’ had the *Bx7+By8* allele at the *Glu-B1* locus (Figure 2.2 and Table 16).

‘97Emmer19’ showed ω -gliadins 30 and 33, γ -gliadins 44, 46 and 48, and β -gliadin 58 (Figure 3.2 and Table 16). ‘2000EB4’ showed ω -gliadin 35, γ -gliadins 45, 46 and 48, and β -gliadin 58 (Figure 3.2 and Table 16) and ‘X.98.142.17’, ‘X.98.142.18’, and ‘P.01.64.31’ showed similar gliadin bands as seen in the ‘2000EB4’ parent with the absence of γ -gliadin 46 (Figure 3.2 and Table 16). All the emmer-derived breeding lines showed the absence of α -gliadins, except ‘05Emmereg’ series lines which showed α -gliadin 65 (Figure 3.2 and Table 16), derived from the parent ‘AC Navigator’ (Table 16). ‘05Emmereg-03’, ‘05Emmereg-10 and ‘05Emmereg-26’ lines showed ω -gliadins 22 and 35, γ -gliadins 45 and 48, and β -gliadin 58 (Figure 3.2 and Table 16), whereas ‘05Emmereg-01’ showed ω -gliadin 35, γ -gliadins 45 and 46, and β -gliadin 58 (Figure 3.2 and Table 16). CIMMYT translocation lines had LMW-2 banding pattern with associated γ -gliadin 45 bands (Figure 3.1 and Table 16). The LMW-2 banding pattern (γ -gliadin 45) was seen in all the durum wheats tested, with the exception of ‘Stewart-63’ and the USDA-ARS translocation line ‘L252’, which had the LMW-1 banding pattern (γ -gliadin 42 – Figures 3.1 and 3.2; Table 16). All the durum genotypes had ω -gliadins 19 and 35, and γ -gliadins 45 and 48, and with the exception of Stewart-63, which had ω -gliadins 22, 33 and 38 in addition to 19 and 35 and γ -gliadin 42 instead of 45 (Figure 3.1 and Table 16). All the durum genotypes had β -gliadins 52 and 56 (Figure 3.1 and Table 16).

Table 17. Contrast (single degree of freedom) between loaf volumes (genotypes evaluated in 2005) and protein subunit(s) detected in tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation lines and emmer-derived breeding lines).

	<i>Glu-A1</i>	<i>Glu-B1</i>	Beta-gliadins	Difference in LV ^a estimate	SED ^b
Tetraploid wheats ^d	<i>1Ax1</i> vs. Null			83*	34.0
	Null vs. <i>Dx5+Dy10</i> ^c			272**	36.7
	<i>1Ax1</i> vs. <i>Dx5+Dy10</i>			354**	41.6
Tetraploid wheats		<i>Bx14+By15</i> , 9* vs. <i>Bx6+By8</i>		155	ns
		<i>Bx14+By15</i> , 9* vs. <i>Bx7+By8</i>		179	ns
		<i>Bx6+By8</i> vs. <i>Bx7+By8</i>		24	ns
Excluding USDA-ARS translocation lines ^e		<i>Bx14+By15</i> , 9* vs. <i>Bx6+By8</i>		65	ns
		<i>Bx14+By15</i> , 9* vs. <i>Bx7+By8</i>		179**	48.9
		<i>Bx6+By8</i> vs. <i>Bx7+By8</i>		114**	35.3
Tetraploid wheats			58 vs. 52,56	167**	57.4
			52,56 vs. 56	91	ns
			58 vs. 56	257	ns
Excluding USDA-ARS translocation lines			58 vs. 52,56	64**	20.1
			52,56 vs. 56	193**	40.3
			58 vs. 56	257**	40.3

* $P < 0.05$ ** $P < 0.01$ ns non significant at $P = 0.05$

^aLV = Loaf Volume

^bSED = Standard Error of the Difference

^cTranslocation of *Dx5+Dy10* from 1D chromosome of hexaploid wheat into 1A chromosome of durum wheat

^dTetraploid wheats = Analysis of all tetraploid genotypes grown during 2005 growing season.

^eExcluding USDA-ARS translocation lines = Analysis of all genotypes grown during 2005 growing season, except USDA-ARS 1AS.1AL-1DL translocation lines

Table 18. Contrast (single degree of freedom) between loaf volumes (genotypes evaluated in 2006) and protein subunit(s) detected in tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation lines and emmer-derived breeding lines).

	<i>Glu-A1</i>	<i>Glu-B1</i>	Beta-gliadins	Difference in LV ^a estimate	SED ^b
Tetraploid wheats ^d	<i>1Ax1</i> vs. Null			34*	15.8
	Null vs. <i>Dx5+Dy10</i> ^c			281**	18.9
	<i>1Ax1</i> vs. <i>Dx5+Dy10</i>			315**	19.7
Excluding USDA-ARS translocation lines ^e	<i>1Ax1</i> vs. Null			34*	16.4
	Null vs. <i>Dx5+Dy10</i>			-	-
	<i>1Ax1</i> vs. <i>Dx5+Dy10</i>			-	-
Tetraploid wheats		<i>Bx14+By15</i> , 9* vs. <i>Bx6+By8</i>		103	ns
		<i>Bx14+By15</i> , 9* vs. <i>Bx7+By8</i>		84	ns
		<i>Bx6+By8</i> vs. <i>Bx7+By8</i>		-19	ns
Excluding USDA-ARS translocation lines ^e		<i>Bx14+By15</i> , 9* vs. <i>Bx6+By8</i>		23	ns
		<i>Bx14+By15</i> , 9* vs. <i>Bx7+By8</i>		84**	25.8
		<i>Bx6+By8</i> vs. <i>Bx7+By8</i>		61*	21.8
Tetraploid wheats			58 vs. 52,56	150**	46.3
Excluding USDA-ARS translocation lines			58 vs. 52,56	53**	12.3

* $P < 0.05$ ** $P < 0.01$ ns non significant at $P = 0.05$

^aLV = Loaf Volume

^bSED = Standard Error of the Difference

^cTranslocation of *Dx5+Dy10* from 1D chromosome of hexaploid wheat into 1A chromosome of durum wheat

^dTetraploid wheats = Analysis of all tetraploid genotypes grown during 2005 growing season.

^eExcluding USDA translocation lines = Analysis of all genotypes grown during 2005 growing season, except USDA-ARS 1AS.1AL-1DL translocation lines

Table 19. Contrast (single degree of freedom) between loaf volumes (combined data from both the 2005 and 2006 years) and protein subunit(s) detected in tetraploid wheats (durum, emmer, 1AS.1AL-1DL translocation lines and emmer-derived breeding lines).

	<i>Glu-A1</i>	<i>Glu-B1</i>	Beta-gliadins	Difference in LV ^a estimate	SED ^b
Tetraploid wheats ^d	<i>1Ax1</i> vs. Null			52**	17.9
	Null vs. <i>Dx5+Dy10</i> ^c			282**	19.2
	<i>1Ax1</i> vs. <i>Dx5+Dy10</i>			333**	21.0
Excluding USDA-ARS translocation lines ^e	<i>1Ax1</i> vs. Null			52**	17.5
	Null vs. <i>Dx5+Dy10</i>			-	-
	<i>1Ax1</i> vs. <i>Dx5+Dy10</i>			-	-
Tetraploid wheats		<i>Bx14+By15</i> , 9* vs. <i>Bx6+By8</i>		133	ns
		<i>Bx14+By15</i> , 9* vs. <i>Bx7+By8</i>		96	ns
		<i>Bx6+By8</i> vs. <i>Bx7+By8</i>		-37	ns
Excluding USDA-ARS translocation lines ^e		<i>Bx14+By15</i> , 9* vs. <i>Bx6+By8</i>		39	ns
		<i>Bx14+By15</i> , 9* vs. <i>Bx7+By8</i>		96**	30.4
		<i>Bx6+By8</i> vs. <i>Bx7+By8</i>		56*	23.8
Tetraploid wheats			58 vs. 52,56	157*	59.3
Excluding USDA-ARS translocation lines			58 vs. 52,56	57**	15.3

* $P < 0.05$ ** $P < 0.01$ ns non significant at $P = 0.05$

^aLV = Loaf Volume

^bSED = Standard Error of the Difference

^cTranslocation of *Dx5+Dy10* from 1D chromosome of hexaploid wheat into 1A chromosome of durum wheat

^dTetraploid wheats = Analysis of all tetraploid genotypes grown during 2005 growing season.

^eExcluding USDA translocation lines = Analysis of all genotypes grown during 2005 growing season, except USDA-ARS 1AS.1AL-1DL translocation lines

16). ‘AC Superb’ differed in ω - and β -gliadins from the other two bread wheat checks, carrying ω -gliadins 19, 30 and β -gliadin 56 (Figure 3.1 and Table 16).

4.6 Contrast Analysis of Loaf Volumes and Protein Subunit(s)

The correlations between loaf volume and rheological properties suggested that gluten strength and dough extensibility were most associated with variation in LV among the durum and ‘97Emmer19’ and the emmer-derived breeding lines (Tables 14 and 15) and variation in the gluten subunits was largely responsible for differences in rheological properties (see sections 4.3 and 4.5). Using single degree of freedom, contrasts (ANOVA) for the 2005- (Table 17) and 2006- (Table 18) grown genotypes and for the combined analysis of composite samples (Table 19) were conducted to determine whether variation in gliadin and glutenin subunit(s) composition between lines (Table 16) could explain differences in LV potential.

Contrast analysis revealed that allelic variation at *Glu-A1* was significantly associated with variation in LV ($P < 0.01$) (Tables 17, 18, and 19). The null allele, present in all of the durum wheat checks, produced LVs 83 and 34 cc smaller than genotypes carrying the *1Ax1* allele at *Glu-A1* locus in 2005 and 2006, respectively (Tables 17, 18 and 19). Averaged over genotypes evaluated in both years, the difference was 52 cc (Table 19). This indicates that the *1Ax1* allele seen in some of the emmer-derived breeding lines had a significant effect on increasing loaf volumes as compared to the null allele seen in durum wheat checks.

When the USDA-ARS translocation lines were considered for the contrast analysis, none of the protein subunits seen at the *Glu-B1* locus showed a significant association with loaf volume (Tables 17, 18, and 19). However, exclusion of USDA-ARS translocation lines from the contrast analysis revealed that genotypes carrying *Bx14+By15* in combination with 9* (Table 16) produced greater LV than genotypes carrying *Bx6+By8* or *Bx7+By8* in 2005 and 2006 (Tables 17 and 18). This suggests that the *Bx14+By15* alleles in combination with 9* present in ‘97Emmer19’ and in some of the emmer-derived breeding lines (Table 16) had a positive effect on LV. The contrast was significant in 2005 ($P < 0.01$; Table 17), 2006 ($P < 0.05$; Table 18), and with the combined analysis ($P < 0.05$; Table 19).

The effect of gliadins on dough rheological properties is less understood in durum wheat (Joppa et al., 1983; Gupta and Shepherd, 1987). In 2005, the contrast analysis with the USDA-ARS translocation lines removed from the data set showed that β -gliadin-58 (seen in some emmer-derived breeding lines) had a significant, positive effect on LV compared to genotypes carrying β -gliadin 52 and 56 (Tables 17, 18, and 19). The CIMMYT lines were only evaluated in 2005 and these lines carried only β -gliadin 56 (Table 16). Contrasts between β -gliadin 52 and 56 and β -gliadin 56 carrying genotypes revealed that, on average, genotypes carrying β -gliadin 52 and 56 had 193 cc higher LV than those only carrying β -gliadin 56. These results suggest that β -gliadin 56 has a negative effect on LV in durum wheat, but this effect can be masked by the presence of β -gliadin 52.

5. General Discussion and Conclusions

Durum wheat grain quality is complex and is a function of its end-use (Troccoli et al., 2000). Although durum wheat is used predominantly for pasta products, its use for bread-making is increasing, particularly in Mediterranean countries. The bread-making properties of wheat depend on several factors including wheat variety (Shoup et al., 1966), environmental and soil conditions (Lloveras et al., 2001), the process used to mill the wheat into flour/semolina (Pomeranz et al., 1970), and the chemical composition of the flour/semolina (David and Ainsworth, 1994; Peña et al., 1995; Raciti et al., 2003). A clear understanding of the physical, chemical and rheological factors that influence the bread-making quality of durum wheat would allow for breeding and development of durum wheat varieties with improved bread-making quality. The objective of this thesis was to assess the rheological factors contributing to enhanced baking quality in durum wheat. In addition, physical (test weight, kernel weight and grain hardness) and chemical properties (grain and flour/semolina protein concentration, SDS sedimentation volume, falling number, yellow pigment concentration and gluten strength) were also assessed as durum wheat cultivars with better bread-making quality must also possess quality attributes important to pasta production, recognizing that a compromise between pasta and bread-making quality factors may be required.

In general, the breeding targets for high baking quality wheat include a balance between dough elasticity and extensibility to ensure good sheeting properties, and the ability to expand and hold gas during the baking process (Dexter, 1993; Edwards et al., 2001). Unlike bread, pasta is an extruded product and stronger gluten produces a better quality pasta product (Edwards et al., 2001). In addition dough extensibility is not a prerequisite for good pasta (Feillet and Dexter, 1996), and breeding efforts to develop stronger, inextensible gluten for pasta end-use have resulted in durum genotypes that contain tenacious, inextensible gluten (Quaglia, 1988; Rao et al., 2000; Ammar et al., 2000; Edwards et al., 2001). Unfortunately, tenacious inextensible gluten contributes to poor bread-making quality (Redaelli et al., 1997; Liu et al., 1996; Ammar et al., 2000; Edwards et al., 2001, 2007; Sapirstein et al., 2007) and suggests that an appropriate

balance of gluten strength and dough extensibility will be required to achieve durum wheat cultivars with good pasta and bread baking quality.

A number of strategies have been suggested to improve the bread-making quality of durum wheat. The *Dx5+Dy10* HMW glutenin subunits on chromosome 1D are known to contribute to enhanced baking quality in hexaploid wheats (Payne et al., 1984, 1987, Shewry et al., 1992, 1997) and transfer of these subunits into tetraploid wheat genetic backgrounds has been pursued as one strategy to improve the bread-making quality of durum wheat (Vitelozzi et al., 1997; Joppa et al., 1998; Blanco et al., 2002; Lukaszewski, 2003). The USDA-ARS 1AS.1AL-1DL translocation lines used in this study were confirmed to carry the *Dx5+Dy10* subunits (Figure 2.2). Of these, ‘L252’ had the LMW-1 (γ -42) banding pattern derived from its recurrent parent ‘Langdon’, whereas the 1B-encoded gliadins seen in ‘L092’, ‘S99B33’, and ‘S99B34’ (γ -45) are from ‘Renville’ (Xu et al., 2005). Consistent with a previous study (Klindworth et al., 2005), the addition of the *Dx5+Dy10* subunits resulted in very strong, inextensible gluten as evidenced by high alveograph *P* values and low alveograph *L* values (Table 11). The USDA-ARS translocation lines tested in this study did not exhibit improved loaf volumes and dough mixing characteristics compared to the durum wheat check varieties (Table 12), and had lower loaf volumes than ‘Stewart-63’ which was included as a negative control in this study. Contrast analysis (Tables 17-19) revealed that, on average, these lines produced loaf volumes 280 cc lower than the remaining tetraploid wheat genotypes (durum and emmer-derived wheats) evaluated. In addition, these lines produced poor loaves, with unappealing loaf shape and crumb structure. The poor baking quality was likely due to the inextensible dough, obtained from these lines (Table 11) which would have limited dough expansion during the fermentation stage of the baking process. These lines displayed very tight dough with no elasticity and as a result the dough handling properties during the baking process were poor. The USDA-ARS translocation lines, might, because of the very strong gluten require mixing at higher speeds than that possible in the GRL 200 mixer used in this study for effective dough development. However, the data presented here suggests that inextensible dough in combination with high gluten strength in these lines limits their use for bread-making. Alternative dough

additives like L-cysteine hydrochloride (acts as reducing agent) may act to improve the mixing characteristics and loaf volumes of USDA-ARS translocation lines.

Various researchers (D'Ovidio, 1993; Ruiz and Carrillo, 1995; Vazquez et al., 1996; Porceddu et al., 1998; Masci et al., 1998; Sissons et al., 2005; Edwards et al., 2007a) have reported that the LMW-2 (γ -45) banding pattern is responsible for endowing semolina with better rheological properties. In this study, the genotypes carrying the LMW-2 banding pattern exhibited greater gluten strength and superior dough rheological properties than those carrying the LMW-1 (γ -42) banding pattern. Interestingly, 'L252', which carried the LMW-1 (γ -42) banding pattern, showed consistently better mixing characteristics (longer mixing time) and higher LV than 'L092', which carried the LMW-2 (γ -45) banding pattern (Tables 12 and 16), suggesting that γ -42 may have compensated for negative effect of *Dx5+Dy10* translocation. 'L252' displayed greater dough extensibility (higher alveograph *L*) in both composite samples (Table 11) and the highest alveograph *W* values in both years of composite testing (Table 11), both factors reported to be important to the bread-making quality of durum wheat (Nash et al., 2006; Edwards et al., 2007). Alternatively, 'L252' had higher grain and semolina protein concentrations and higher dry gluten content compared to 'L092' (Tables 4, 6, and 8), which could be the reason for the higher loaf volumes, despite no significant correlations being detected in this study between loaf volume and the two traits (Tables 14 and 15). However, elevated protein concentration has been associated with elevated LV in durum wheat in other studies, using both short fermentation (Dexter et al., 1994, 1998; Marchylo et al., 2001; Sapirstein et al., 2007) and long fermentation processes (Boyacioglu and D'Appolonia, 1994; Boggini et al., 1995; Peña, 2000; Palumbo et al., 2000; Sapirstein et al., 2007).

A second strategy to improve the bread-making quality of durum wheat is to identify wild relatives of durum wheat that may contain variation for enhanced baking quality. In this study, '97Emmer19', a wild relative of durum wheat, displayed improved loaf volume over Canadian durum wheat check cultivars (Table 12), confirming earlier results (Schlichting et al., 2002). In this research, we evaluated breeding lines derived from crossing '97Emmer19' to adapted durum wheat germplasm, and some of the emmer-derived breeding lines ('2000EB4', 'X.98.142.17', 'P.01.64.31', '05Emmereg-

03', and '05Emmereg-26') exhibited baking performance similar to the '97Emmer19' (Table 12). These results suggest that factors responsible for the superior bread-making quality in '97Emmer19' are heritable and could be the target of breeding programs to develop dual-purpose durum cultivars. However, despite having improved loaf volumes, the majority of emmer-derived breeding lines possessed dough rheological properties different than '97Emmer19' in terms of gluten strength, farinograph and alveograph parameters (Tables 8, 10, and 11), suggesting that an appropriate balance of these factors is more critical to improving bread-making quality. There is general agreement on the poor gluten quality (i.e., weak gluten strength and low dough extensibility) of wild emmer accessions as a consequence of the absence of gliadin fractions γ -42 and γ -45 (Galterio et al., 1994, 1998, 2000; Fares et al., 2002). In this study, the LMW-1 (γ -42) and LMW-2 (γ -45) banding patterns are lacking in '97Emmer19' (Figure 2.1, Table 16). The absence of γ -42 and γ -45 in wild emmer accession '97Emmer19' is in accordance with earlier observations by Galterio et al. (1994) and Oak et al. (2002a) in Italian and Indian *dicoccum* germplasms, respectively. As suggested by Galterios et al. (2001), the improved gluten strength and high dough extensibility (L) seen in genotypes such as some '97emmer19'-derived breeding lines seems to be related to the presence of the LMW-2 subunits associated with the ω -35 and γ -45 gliadin fractions derived from the durum parent (in this study either 'WB881' or 'AC Navigator') used in the breeding program.

In the present study, dough extensibility (L) was essentially unrelated to gluten strength as evidenced by the lack of correlation with either gluten index (GI) or alveograph deformation energy (W), although dough extensibility L was correlated with SDS sedimentation volumes when USDA-ARS translocation lines were not included in the correlation analysis (Tables 14 and 15). These results suggest that gluten strength and dough extensibility (L) are the most important traits responsible for the enhanced baking quality seen in emmer-derived breeding lines. Ammar et al. (2000), Edwards et al. (2001) and Rao et al. (2001) also suggested that these two factors were most important to the enhanced baking quality of durum wheat. For bread-making, high dough extensibility (L) is favored and is related to the need for gas cells within the fermenting dough to be

extended without rupture or with minimal rupture during the proofing and early stages of baking (Larroque et al., 1999; Anderssen et al., 2004).

Although correlation analysis confirmed that strong gluten and more extensible dough were important to LV potential in durum wheat, '97Emmer19' displayed both poor gluten strength and little dough extensibility. These results suggest that an appropriate balance of gluten strength and extensibility are important to achieve higher loaf volumes in durum wheat since both factors indirectly characterize the extent of the expansion that will occur during the expansion of the gas bubbles (Bloksma, 1990; Eliasson and Larsson, 1993; Kokelaar, 1994). The fact that '97Emmer19' possessed low alveograph *P* and *L* (Table 11), but high loaf volume (Table 12) may suggest that with weaker gluten, extensible dough is not a requirement for dough expansion during proofing. In contrast, 'Arcola' displayed a higher alveograph *P* than '97Emmer19' coupled with high alveograph *L* and the dough exhibited relatively poor dough handling properties and poor loaf volume when compared to '97Emmer19'. These results suggest that at higher *P* values, more extensible dough is required to achieve elevated loaf volume. In support of this hypothesis, the durum wheat checks, 'Commander', 'AC Navigator' and 'DT724' had high *P* values, but inextensible dough (Table 11). In addition, 'WB881' and '2000EB4' showed higher gluten strength coupled with more extensible dough and both lines produced higher loaf volumes compared to the durum wheat checks (Tables 11 and 12). Although these lines did not produce loaf volumes similar to that of '97Emmer19', both lines did have lower semolina protein and significantly lower dry gluten (Table 8). Perhaps if compared at equivalent protein concentrations, '2000EB4' and 'WB881' may have performed as well as '97Emmer19'.

Of the emmer-derived breeding lines, only '05Emmereg-03' produced loaf volumes statistically equivalent to the bread wheat check 'AC Superb' (Table 12). However, the bread wheats possessed lower resistance to extension (*P*) combined with high extensibility (*L*), both factors which prevent gas cells from collapsing under the weight of the dough during proofing (Larroque et al., 1999). Nash et al. (2006) reported that in durum, the *Glu-3/Gli-1* complex on chromosome 1B has a positive effect on dough strength (alveograph *P*) but a corresponding negative effect on dough extensibility (alveograph *L*). Thus, it appears that the same locus that confers tolerance to mixing

(dough strength) also causes low dough extensibility. In the durum wheat lines evaluated in this study, extensibility was negatively correlated ($r = -0.47$; $P < 0.05$) with alveograph P (Table 14). This presents a challenge for breeding durum wheat cultivars suitable for bread-making, in that selection for strength may equate to selection for poor dough extensibility (L). This is particularly true since most durum breeding programs are selecting for the high alveograph P values in demand by the pasta industry (Dexter and Marchylo, 1996). However, the linkage between P and L is not perfect, as ‘2000EB4’ and ‘WB881’ showed good gluten strength, and extensible dough. Since ‘WB881’ is a registered variety in the USA, the extensibility of dough produced by this variety must not negatively influence pasta quality. Samaan et al. (2006) have shown that the higher extensibility readings (using the Extensograph) in Syrian spring durum wheat genotypes were associated with increased pasta firmness ($P < 0.05$) and optimum-cooking time of pasta. However, reports on the negative or positive relationship between high dough extensibility and pasta quality are lacking, as most of the registered Canadian durum cultivars are not extensible.

Since differences in rheological properties were associated with variation in loaf volume, the gluten subunits present in each of the lines was determined and correlated to variation in loaf volume. ‘97Emmer19’ and the majority of its progeny expressed the *1Ax1* allele at the *Glu-A1* locus. Contrast analysis (after exclusion of the USDA-ARS translocation lines) revealed that, on average, lines carrying the *1Ax1* allele had loaf volumes 52 cc higher than those lacking the *1Ax1* allele (Table 19). In previous studies that evaluated alleles at the *Glu-A1* locus, genotypes carrying *Glu-A1a** allele (*1Ax1*) or *Glu-A1b* allele (*1Ax2**) were noted to have better loaf volumes compared to genotypes carrying the *Glu-A1c* (null) allele (Halford et al., 1992; Branlard and Dardevet, 1985a, 1985b; Payne, 1987; Nieto-Taladriz et al., 1994; Sontag-Strohm et al., 1996). Nearly all of the durum wheat checks, including ‘05Emmereg-03’ carried the null allele at *Glu-A1* locus (Table 16) which has been associated with inextensible dough and poor bread-making quality in bread wheat (Payne, 1987; Nieto-Taladriz et al., 1994; Sontag-Strohm et al., 1996). However, in 2006 testing, ‘05Emmereg-03’ had the highest LV of the tetraploid wheats and was numerically similar to ‘AC Superb’ (Table 12), despite carrying the null allele at *Glu-A1*. However, ‘97Emmer19’, ‘2000EB4’ and ‘05Emmereg-

03' carried the *Bx14+By15* allele in combination with the 9* allele at *Glu-B1* (Table 16), suggesting that this allele may also be contributing to the elevated loaf volumes seen in those lines. Contrast analysis revealed that the differences in loaf volume among genotypes carrying *Bx14+By15* in combination with 9* allele vs. those carrying the *Bx6+By8* (the majority of durum wheat checks; Table 16) was 39 cc, but this difference was not statistically significant (Table 19). However, this analysis did not account for the fact that many of the emmer-derived breeding lines carried *1Ax1* at the *Glu-A1* locus, which was associated with increased loaf volume. Unfortunately, contrast analysis to determine which locus (either *Glu-A1* locus carrying *1Ax1* allele or *Glu-B1* locus carrying *Bx14+By15* in combination with 9*) was showing greater association with increased loaf volume could not be conducted due to the lack of degrees of freedom (i.e. too few genotypes with appropriate combinations of *1Ax1* and *Bx14+By15* in combination with 9* were evaluated over both years). Given the inconsistency among the emmer-derived breeding lines, the influence of these subunits on the rheological properties of the dough could not be determined.

In this study, for '97Emmer19' and all of the emmer-derived breeding lines carried β -gliadin 58, contrast analysis revealed that averaged over composite samples, genotypes carrying this allele had loaf volumes 157 cc larger than the durum wheat genotypes lacking the allele (Table 19). All of the emmer-derived breeding lines chosen for this study were selected from breeding populations developed from crossing '97Emmer19' to 'AC Navigator', 'WB881' or 'AC Avonlea' (Table 1), and both 'AC Navigator' and 'WB881' carry β -gliadins 52 and 56 (Table 16). 'AC Avonlea' is a parent of 'Strongfield' (Clarke et al., 2006b), and the latter also carries β -gliadins 52 and 56. The emmer-derived breeding lines were developed with elevated loaf volume as a selection criterion, and the fact that all of these lines carried the β -gliadin 58 allele suggests that this gliadin subunit may be associated with elevated LV, and could be the target for indirect selection for breeding programs improving durum wheat for bread-making quality.

This study focused on assessing the bread-making quality of durum wheat. However, for development of a dual-purpose cultivar, traits important to the pasta industry must not be compromised. Durum wheat breeders targeting cultivars for pasta

must ensure that cultivars possess high test weight (TWT), high kernel weight (KWT), high falling number (FN), high grain and semolina protein concentrations, high yellow pigment concentration (YPC) and semolina milling yield, and good pasta making and cooking quality. Obviously, ‘97Emmer19’ is not a good candidate for dual-purpose end-use as it showed low TWT and KWT, poor FN and low YPC (Tables 3 and 5). Although ‘97Emmer19’ consistently expressed high grain and semolina protein concentrations (Tables 4 and 6), it is likely the elevated protein is due to its small seed size (Table 3). In contrast, improved quality for many of these pasta-related quality traits are seen in some of the emmer-derived breeding lines:

- a. ‘2000EB4’ showed high average GPC (14.3%; Table 4), high average FN (470 sec; Table 5), high average TWT (78.9 kg hL⁻¹; Table 3) and high average KWT (39.7 g; Table 3).
- b. ‘X.98.142.17’ showed high average GPC (13.1%; Table 4), high average milling yield (66.4%; Table 6) and high average KWT (43.7 g; Table 3).
- c. ‘P.01.64.31’ showed high average GPC (13.0%; Table 4), high average milling yield (66.4%; Table 6), high average YPC (6.4 mg kg⁻¹; Table 5), high average TWT (80.2 kg hL⁻¹; Table 3) and high average KWT (47.2 g; Table 3).
- d. ‘P.01.64.39’ showed high average GPC (14.0%; Table 4), high average YPC (7.2 mg kg⁻¹; Table 5), high average TWT (80.3 kg hL⁻¹; Table 3) and high average KWT (44.2 g; Table 3).
- e. ‘05Emmereg-03’ showed high average GPC (15.4%; Table 4), high average FN (496 sec; Table 5) and high average KWT (45.3 g; Table 3).
- f. ‘05Emmereg-26’ showed high average GPC (13.3%; Table 4), high average FN (494 sec; Table 5), high average milling yield (66.5%; Table 6), high average YPC (6.9 mg kg⁻¹; Table 5) and high average KWT (51.2 g; Table 3).

These results suggest that the heritable variation for loaf volume is independent of these traits and breeding efforts can be continued to further improve pasta-related quality

traits. However, only pasta quality predictors were assessed in this study, and the material generated in this thesis should be used to study pasta quality in more detail to ensure no negative effects of improved bread-making on pasta quality. The data presented in this thesis also support the possibility of introgressing economically important durum traits like gluten strength into the emmer genotypes while preserving the morphological, functional and botanical characteristics of emmer. The emmer-derived breeding lines in this study have shown agronomically superior performance over the emmer parent (data not shown).

Conclusions

1. '97Emmer19' has superior baking quality (loaf volume) over the durum wheat checks 'Strongfield', 'WB881', and 'AC Navigator' and performed similar to the Canadian Western Red Spring (CWRS) bread wheat check 'AC Superb'. The variation for bread-making quality is heritable.
2. A balance of gluten strength and dough extensibility (*L*) is an important requirement when breeding durum wheats for improved baking quality. Data presented here suggest that strong gluten genotypes, a current breeding target for durum made into pasta, will require more extensible dough to achieve higher loaf volumes. However, this balance may be difficult to achieve and could imply that the loaf volumes of durum wheats could be increased, but the loaf volume potential as seen in CWRS bread wheats may not be achieved.
3. The superior baking quality seen in '97Emmer19' and some of the emmer-derived breeding lines can be attributed to the gliadin and glutenin protein subunits. Results suggest that the HMW-GS *1Ax1* at the *Glu-A1* locus and *Bx14+By15*, 9* at the *Glu-B1* locus and β -gliadin 58 are associated with elevated LV in the emmer-derived breeding lines. These subunits could potentially be used as protein markers to indirectly select for improved baking quality, although our results suggest that these markers would not be perfect and bake tests would still be required to assess phenotypic worth.
4. Despite carrying the *Glu-D1d* (HMW-GS pair *1Dx5+1Dy10*) allele, USDA-ARS 1AS.1AL-1DL translocation lines exhibited poor baking quality compared to durum wheat checks and emmer-derived breeding lines. To enhance the baking quality of durum wheat, the use of 1AS.1AL-1DL translocation lines from 'Renville' and 'Langdon' tetraploid wheats background is not recommended.
5. Breeding to combine bread-making quality with traits important to the pasta industry appears to be feasible, but more work is needed to properly assess the pasta quality of some emmer-derived breeding lines, particularly the AC

Navigator//2000EB4/AC Navigator and 2000EB4/AC Avonlea derived breeding lines.

6. Future Work

1. More work is required to determine the pasta quality of those emmer-derived breeding lines exhibiting improved baking quality ('2000EB4', 'X.98.124.17', 'P.01.64.31', '05Emmereg-03', and '05Emmereg-26'). This is required to determine if the pasta quality of these lines is negatively influenced by the factors contributing to improved loaf volume.
2. In the present study, the association of increased loaf volumes with *1Ax1* (*Glu-A1*), *Bx14+By15* in combination with 9* (*Glu-B1*) and β -gliadin 58, was based on a small sample size. A large, segregating population derived from crossing lines with and without these subunits (e.g., '2000EB4' x 'AC Navigator'; Table 16) should be evaluated in multiple environments to determine the effects of each of these subunits alone and in combination on loaf volume.

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8. Appendices

Appendix A1. Summary of ANOVA for Test Weight (kg hL⁻¹) and Kernel Weight (g) for 2005 and 2006 grown genotypes at Goodale, Kernen, and Seed Farm environments and data averaged over environments.

Quality parameter	Location	Year	ANOVA	Random effect variance estimate ($\alpha = 0.05$)	Fixed Effect F-value
Test weight (kg hL ⁻¹)	GDL ^a	2005	Genotype	-	31.72**
			Replication	0.06 ^{ns}	-
			Residual	0.40**	-
		2006	Genotype	-	17.96**
			Replication	0.04 ^{ns}	-
			Residual	0.52**	-
	KER ^b	2005	Genotype	-	26.27**
			Replication	0.00	-
			Residual	0.53**	-
		2006	Genotype	-	13.57**
			Replication	0.12 ^{ns}	-
			Residual	0.34**	-
	SF ^c	2005	Genotype	-	11.48**
			Replication	0.01 ^{ns}	-
			Residual	1.72**	-
2006		Genotype	-	13.52**	
		Replication	0.04 ^{ns}	-	
		Residual	0.46**	-	
Data averaged over environments			Genotype	-	7.57**
			Year	5.20 ^{ns}	-
			Location	0.00	-
			Location*Year	0.47 ^{ns}	-
			Replication(location*year)	0.05 ^{ns}	-
			Location*Genotype	0.00	-
			Genotype*Year	0.68**	-
			Location*Genotype*Year	0.32**	-
		Residual	0.58**	-	
1000 Kernel Weight (g)	GDL	2005	Genotype	-	22.73**
			Replication	0.00	-
			Residual	2.98**	-
		2006	Genotype	-	43.21**
			Replication	0.26 ^{ns}	-
			Residual	1.86**	-
	KER	2005	Genotype	-	23.64**
			Replication	0.04 ^{ns}	-
			Residual	1.80**	-
		2006	Genotype	-	23.94**
			Replication	0.30 ^{ns}	-
			Residual	2.98**	-
	SF	2005	Genotype	-	17.54**
			Replication	0.30 ^{ns}	-
			Residual	3.69**	-
2006		Genotype	-	11.93**	
		Replication	0.25 ^{ns}	-	
		Residual	6.05**	-	
Data averaged over environments			Genotype	-	18.23**
			Year	2.07 ^{ns}	-
			Location	0.00	-
			Location*Year	1.70 ^{ns}	-
			Replication(location*year)	0.09 ^{ns}	-
			Location*Genotype	0.59 ^{ns}	-
			Genotype*Year	1.52*	-
			Location*Genotype*Year	0.86*	-
		Residual	3.14**	-	

^aGDL = goodale location ^bKER = kernen location ^cSF = seed farm location * $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

Appendix A2. Summary of ANOVA for Grain Hardness and Grain Protein Concentration (GPC) for 2005 and 2006 grown genotypes at Goodale, Kernen, and Seed Farm environments and data averaged over environments.

Quality parameter	Location	Year	ANOVA	Random effect variance estimate ($\alpha = 0.05$)	Fixed Effect F-value
Hardness Index	GDL ^a	2005	Genotype	-	85.27**
			Replication	0.22 ^{ns}	-
			Residual	2.33**	-
		2006	Genotype	-	19.57**
			Replication	0.40 ^{ns}	-
			Residual	9.01**	-
	KER ^b	2005	Genotype	-	113.90**
			Replication	0.06 ^{ns}	-
			Residual	1.74**	-
		2006	Genotype	-	80.95**
			Replication	0.00	-
			Residual	2.01**	-
	SF ^c	2005	Genotype	-	22.41**
			Replication	1.89 ^{ns}	-
			Residual	8.07**	-
2006		Genotype	-	10.36**	
		Replication	1.97 ^{ns}	-	
		Residual	11.33**	-	
Data averaged over environments			Genotype	-	19.87**
			Year	25.92 ^{ns}	-
			Location	0.00	-
			Location*Year	2.15 ^{ns}	-
			Replication(location*year)	0.74*	-
			Location*Genotype	0.00	-
			Genotype*Year	4.02*	-
			Location*Genotype*Year	3.03**	-
		Residual	5.96**	-	
Grain protein concentration (13.5% mb)	GDL	2005	Genotype	-	48.78**
			Replication	0.00	-
			Residual	0.13**	-
		2006	Genotype	-	2.64**
			Replication	0.21 ^{ns}	-
			Residual	1.02**	-
	KER	2005	Genotype	-	38.20**
			Replication	0.00	-
			Residual	0.12**	-
		2006	Genotype	-	14.34**
			Replication	0.03 ^{ns}	-
			Residual	0.15**	-
	SF	2005	Genotype	-	7.77**
			Replication	0.21 ^{ns}	-
			Residual	0.64**	-
2006		Genotype	-	1.66 ^{ns}	
		Replication	1.11 ^{ns}	-	
		Residual	1.58**	-	
Data averaged over environments			Genotype	-	5.23**
			Year	0.00	-
			Location	0.00	-
			Location*Year	0.48 ^{ns}	-
			Replication(location*year)	0.26*	-
			Location*Genotype	0.00	-
			Genotype*Year	0.04 ^{ns}	-
			Location*Genotype*Year	0.14**	-
		Residual	0.61**	-	

^aGDL = goodale location ^bKER = kernen location ^cSF = seed farm location
* $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

Appendix A3. Summary of ANOVA for SDS Sedimentation volumes (mL) and Falling Number (sec) for 2005 and 2006 grown genotypes at Goodale, Kernen, and Seed Farm environments and data averaged over environments.

Quality parameter	Location	Year	ANOVA	Random effect variance estimate ($\alpha = 0.05$)	Fixed Effect F-value	
SDS Sedimentation volumes (mL)	GDL ^a	2005	Genotype	-	115.27**	
			Replication	0.00	-	
			Residual	8.63**	-	
		2006	Genotype	-	25.47**	
			Replication	0.80 ^{ns}	-	
			Residual	13.27**	-	
	KER ^b	2005	Genotype	-	244.15**	
			Replication	1.08 ^{ns}	-	
			Residual	5.16**	-	
		2006	Genotype	-	123.45**	
			Replication	0.30 ^{ns}	-	
			Residual	3.41**	-	
	SF ^c	2005	Genotype	-	135.18**	
			Replication	0.73 ^{ns}	-	
			Residual	7.38**	-	
		2006	Genotype	-	16.15**	
			Replication	9.11 ^{ns}	-	
			Residual	18.82**	-	
	Data averaged over environments			Genotype	-	25.80**
				Year	3.63 ^{ns}	-
				Location	0.00	-
			Location*Year	8.19 ^{ns}	-	
			Replication(location*year)	1.53*	-	
			Location*Genotype	2.20 ^{ns}	-	
			Genotype*Year	9.40*	-	
			Location*Genotype*Year	7.02**	-	
		Residual	9.72**	-		
Falling number (sec)	GDL	2005	Genotype	-	8.89**	
			Replication	0.00	-	
			Residual	1129.41**	-	
		2006	Genotype	-	5.87**	
			Replication	0.00	-	
			Residual	697.92**	-	
	KER	2005	Genotype	-	26.67**	
			Replication	0.00	-	
			Residual	649.72**	-	
		2006	Genotype	-	11.49**	
			Replication	0.00	-	
			Residual	489.57**	-	
	SF	2005	Genotype	-	13.57**	
			Replication	22.25 ^{ns}	-	
			Residual	677.39**	-	
		2006	Genotype	-	9.43**	
			Replication	153.31 ^{ns}	-	
			Residual	1413.08**	-	
Data averaged over environments			Genotype	0.00	2.42*	
			Year	4594.49 ^{ns}	-	
			Location	830.38 ^{ns}	-	
			Location*Year	152.41 ^{ns}	-	
			Replication(location*year)	22.17 ^{ns}	-	
			Location*Genotype	0.00	-	
			Genotype*Year	1032.89**	-	
			Location*Genotype*Year	579.73**	-	
		Residual	775.64**	-		

^aGDL = goodale location ^bKER = kernen location ^cSF = seed farm location * $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

Appendix A4. Summary of ANOVA for Yellow Pigment Concentration (mg kg⁻¹) for 2005 and 2006 grown genotypes at Goodale, Kernen, and Seed Farm environments and data averaged over environments.

Quality parameter	Location	Year	ANOVA	Random effect variance estimate (alpha = 0.05)	Fixed Effect F-value
Yellow pigment concentration (mg kg ⁻¹)	GDL ^a	2005	Genotype	-	27.83**
			Replication	0.14 ^{ns}	-
			Residual	0.24**	-
		2006	Genotype	-	98.95**
			Replication	0.00 ^{ns}	-
			Residual	0.06**	-
	KER ^b	2005	Genotype	-	142.10**
			Replication	0.00	-
			Residual	0.05**	-
		2006	Genotype	-	202.14**
			Replication	0.01 ^{ns}	-
			Residual	0.03**	-
	SF ^c	2005	Genotype	-	66.06**
			Replication	0.02 ^{ns}	-
			Residual	0.11**	-
2006		Genotype	-	105.55**	
		Replication	0.00	-	
		Residual	0.06**	-	
Data averaged over environments			Genotype	-	82.76**
			Year	0.05 ^{ns}	-
			Location	0.00	-
			Location*Year	0.29 ^{ns}	-
			Replication(location*year)	0.03*	-
			Location*Genotype	0.00	-
			Genotype*Year	0.04**	-
			Location*Genotype*Year	0.01 ^{ns}	-
		Residual	0.08**	-	

^aGDL = goodale location ^bKER = kernen location ^cSF = seed farm location
* $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

Appendix A5. Summary of ANOVA (combined composite samples data for the 2005 and 2006 growing seasons) for Flour/Semolina Milling Yield (%), Agtron colour (%), Brightness (L*), Redness (a*), Yellowness (b*), Gluten Index (GI), Wet Gluten (WG), Dry Gluten (DG), and Flour/Semolina Protein Concentration.

Quality parameter	Year	ANOVA	Random effect variance estimate (alpha = 0.05)	Fixed Effect F-value
Flour/Semolina Milling Yield (%)	Data averaged over years	Genotype	-	17.27**
		Year	0.60 ^{ns}	
		Year*Genotype	0.34 ^{ns}	
		Residual	0.37**	
Agtron	Data averaged over years	Genotype	-	6.14**
		Year	7.47 ^{ns}	
		Year*Genotype	18.10**	
		Residual	4.21**	
L*	Data averaged over years	Genotype	-	21.21**
		Year	0.17 ^{ns}	
		Year*Genotype	0.00	
		Residual	0.07**	
a*	Data averaged over years	Genotype	-	45.31**
		Year	0.01 ^{ns}	
		Year*Genotype	0.00	
		Residual	0.003**	
b*	Data averaged over years	Genotype	-	239.68**
		Year	0.95 ^{ns}	
		Year*Genotype	0.16**	
		Residual	0.01**	
Gluten Index	Data averaged over years	Genotype	-	141.11**
		Year	5.88 ^{ns}	
		Year*Genotype	5.21*	
		Residual	6.55**	
Wet Gluten	Data averaged over years	Genotype	-	9.19**
		Year	1.06 ^{ns}	
		Year*Genotype	0.17 ^{ns}	
		Residual	4.01**	
Dry Gluten	Data averaged over years	Genotype	-	21.93**
		Year	0.04 ^{ns}	
		Year*Genotype	0.07*	
		Residual	0.10**	
Flour/Semolina Protein concentration	Data averaged over years	Genotype	-	20.57**
		Year	0.01 ^{ns}	
		Year*Genotype	0.07**	
		Residual	0.004**	

* $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

Appendix A6. Summary of ANOVA (combined composite data from 2005 and 2006) for farinograph and alveograph quality parameters.

Quality parameter	Year	ANOVA	Random effect variance estimate (alpha = 0.05)	Fixed Effect F-value
FAB ^a	Data averaged over years	Genotype	-	18.76**
		Year	3.08 ^{ns}	-
		Residual	0.49**	-
MTI ^b	Data averaged over years	Genotype	-	26.18**
		Year	0.00	-
		Residual	38.75**	-
DDT ^c	Data averaged over years	Genotype	-	51.85**
		Year	0.34 ^{ns}	-
		Residual	0.82**	-
TTB ^d	Data averaged over years	Genotype	-	9.86**
		Year	0.00	-
		Residual	17.52**	-
STA ^e	Data averaged over years	Genotype	-	11.67**
		Year	1.91 ^{ns}	-
		Residual	29.35**	-
P ^f	Data averaged over years	Genotype	-	11.02**
		Year	568.36 ^{ns}	-
		Year*Genotype	200.74**	-
		Residual	62.23**	-
L ^g	Data averaged over years	Genotype	-	6.11**
		Year	0.00	-
		Year*Genotype	226.63**	-
		Residual	160.41**	-
P/L ^h	Data averaged over years	Genotype	-	4.67**
		Year	0.61 ^{ns}	-
		Year*Genotype	0.62**	-
		Residual	0.23**	-
W ⁱ	Data averaged over years	Genotype	-	6.34**
		Year	1212.98 ^{ns}	-
		Year*Genotype	2050.47**	-
		Residual	1216.49**	-

* $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

^aFAB = Farinograph water absorption (%) ^bMTI = Farinograph Mixing Tolerance Index (B.U.)
^cDDT = Farinograph dough development time (min) ^dTTB = Farinograph time to breakdown (min)
^eSTA = Farinograph stability (min) ^fP = Alveograph tenacity (1.1 x height) (mm)
^gL = Alveograph extensibility (mm) ^hP/L = Alveograph curve configuration ratio
ⁱW = Alveograph deformation energy (J x 10⁻⁴)

Appendix A7. Summary of ANOVA (combined composite samples data for the 2005 and 2006 growing seasons) for Loaf Volume (cc), Loaf Shape, Crumb Colour, Crumb Structure, and Peak Mixing Time (min).

Quality parameter	Year	ANOVA	Random effect variance estimate (alpha = 0.05)	Fixed Effect F-value
Loaf volume (cc)	Data averaged over years	Genotype	-	40.26**
		Year	35.21 ^{ns}	
		Year*Genotype	511.07 ^{ns}	
		Residual	1565.53**	
Loaf shape (measured on 0-5 scale)	Data averaged over years	Genotype	-	27.20**
		Year	0.003 ^{ns}	
		Year*Genotype	0	
		Residual	0.10**	
Crumb Colour (measured on 0-5 scale)	Data averaged over years	Genotype	-	9.53**
		Year	0.12 ^{ns}	
		Year*Genotype	0.04 ^{ns}	
		Residual	0.10**	
Crumb Structure (measured on 0-5 scale)	Data averaged over years	Genotype	-	42.62**
		Year	0.00	
		Year*Genotype	0.00	
		Residual	0.06**	
Peak Mixing Time (min)	Data averaged over years	Genotype	-	9.84**
		Year	0.47 ^{ns}	
		Year*Genotype	0.30*	
		Residual	0.63**	

* $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

Appendix A8. Summary of Contrast analysis for loaf volumes (genotypes evaluated in 2005) based on protein subunit(s) detected in tetraploid wheats

	<i>Glu-A1</i>	<i>Glu-B1</i>	Beta-gliadin	LV ^a Estimate	F-value
Tetraploid wheats ^c	<i>1Ax1</i>			803	
	Null			721	
	<i>Dx5+Dy10</i> ^b			449	
	Type III test of Fixed Effects				39.74**
Tetraploid wheats		<i>Bx14+By15</i> , 9*		828	
		<i>Bx6+By8</i>		673	
		<i>Bx7+By8</i>		649	
		Type III test of Fixed Effects			1.16 ^{ns}
Excluding USDA-ARS translocation lines ^d		<i>Bx14+By15</i> , 9*		828	
		<i>Bx6+By8</i>		762	
		<i>Bx7+By8</i>		649	
		Type III test of Fixed Effects			7.72**
Tetraploid wheats			58	795	
			52,56	629	
			56	538	
			Type III test of Fixed Effects		5.03*
Excluding USDA-ARS translocation lines			58	795	
			52,56	731	
			56	538	
			Type III test of Fixed Effects		21.76**

* $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

^aLV = Loaf Volume

^bTranslocation of *Dx5+Dy10* from 1D chromosome of hexaploid wheat into 1A chromosome of durum wheat

^cTetraploid wheats = Analysis of all tetraploid genotypes grown during 2005 growing season.

^dExcluding USDA translocation lines = Analysis of all genotypes grown during 2005 growing season, except USDA-ARS 1AS.1AL-1DL translocation lines

Appendix A9. Summary of Contrast analysis for loaf volumes (genotypes evaluated in 2006) based on protein subunit(s) detected in tetraploid wheats.

	<i>Glu-A1</i>	<i>Glu-B1</i>	Beta-gliadin	LV ^a Estimate	F-value
Tetraploid wheats ^c	<i>1Ax1</i>			781	
	Null			747	
	<i>Dx5+Dy10</i> ^b			466	
	Type III test of Fixed Effects				144.24**
Tetraploid wheats		<i>Bx14+By15</i> , 9*		788	
		<i>Bx6+By8</i>		685	
		<i>Bx7+By8</i>		704	
		Type III test of Fixed Effects			0.81 ^{ns}
Excluding USDA-ARS translocation lines ^d		<i>Bx14+By15</i> , 9*		788	
		<i>Bx6+By8</i>		765	
		<i>Bx7+By8</i>		704	
		Type III test of Fixed Effects			5.53*
Tetraploid wheats			58	785	
			52,56	635	
			Type III test of Fixed Effects		10.45**
Excluding USDA-ARS translocation lines			58	785	
			52,56	732	
			Type III test of Fixed Effects		18.42**

* $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

^aLV = Loaf Volume

^bTranslocation of *Dx5+Dy10* from 1D chromosome of hexaploid wheat into 1A chromosome of durum wheat

^cTetraploid wheats = Analysis of all tetraploid genotypes grown during 2005 growing season.

^dExcluding USDA translocation lines = Analysis of all genotypes grown during 2005 growing season, except USDA-ARS 1AS.1AL-1DL translocation lines

Appendix A10. Summary of Contrast analysis for loaf volumes (combined data from 2005 and 2006) based on protein subunit(s) detected in tetraploid wheats.

	<i>Glu-A1</i>	<i>Glu-B1</i>	Beta-gliadin	LV ^a Estimate	F-value
Tetraploid wheats ^c	<i>1Ax1</i>			790	
	Null			738	
	<i>Dx5+Dy10</i> ^b			456	
	Type III test of Fixed Effects				146.01**
Tetraploid wheats		<i>Bx14+By15</i> , 9*		800	
		<i>Bx6+By8</i>		667	
		<i>Bx7+By8</i>		704	
		Type III test of Fixed Effects			0.81 ^{ns}
Excluding USDA-ARS translocation lines ^d		<i>Bx14+By15</i> , 9*		800	
		<i>Bx6+By8</i>		760	
		<i>Bx7+By8</i>		704	
		Type III test of Fixed Effects			5.05*
Tetraploid wheats			58	788	
			52,56	632	
			Type III test of Fixed Effects		6.97*
Excluding USDA-ARS translocation lines			58	788	
			52,56	732	
			Type III test of Fixed Effects		13.68**

* $P < 0.05$ ** $P < 0.01$ ^{ns} non significant at $P = 0.05$

^aLV = Loaf Volume

^bTranslocation of *Dx5+Dy10* from 1D chromosome of hexaploid wheat into 1A chromosome of durum wheat

^cTetraploid wheats = Analysis of all tetraploid genotypes grown during 2005 growing season.

^dExcluding USDA translocation lines = Analysis of all genotypes grown during 2005 growing season, except USDA-ARS 1AS.1AL-1DL translocation lines

Appendix B1. Nomenclature of HMW-GS alleles in bread and durum wheat, proposed by Payne and Lawrence (1983).

Locus	Allele	Subunit(s)
Bread wheat		
<i>Glu-A1</i>	<i>Glu-A1a</i> *	1
	<i>Glu-A1b</i>	2*
	<i>Glu-A1c</i>	Null
<i>Glu-B1</i>	<i>Glu-B1b</i>	7+8
	<i>Glu-B1c</i>	7+9
	<i>Glu-B1d</i>	6+8
	<i>Glu-B1f</i>	13+16
	<i>Glu-B1i</i>	17+18
<i>Glu-D1</i>	<i>Glu-D1a</i>	2+12
	<i>Glu-D1d</i>	5+10
Durum wheat		
<i>Glu-B1</i>	<i>Glu-B1a</i>	7
	<i>Glu-B1b</i>	7+8
	<i>Glu-B1d</i>	6+8
	<i>Glu-B1e</i>	20
	<i>Glu-B1f</i>	13+16
	<i>Glu-B1h</i>	14+15

Appendix C1. Molecular weight of high molecular weight protein subunits (in KDa) of Canadian registered wheat cultivars (proposed by Ng and Bushuk, 1988b).

Protein subunit	Molecular weight in KDa
1	149
½*	141
2*	136
2	134
5	128
6	121
7	115
20	113
14	112
15	107
8	101
9	95.7
10	92.1
12	91.2