A STUDY OF THE GENETICS AND PHYSIOLOGICAL BASIS OF GRAIN PROTEIN CONCENTRATION 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 Doctor of Philosophy
In the Department of Plant Sciences
University of Saskatchewan
Saskatoon, Saskatchewan

By
Suprayogi

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements of a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised this thesis work or, in their absence, by the Head of the Department or the Dean of the College in which this thesis work was done. It is understood that any copying or publication or use of this thesis or part thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Plant Sciences
51 Campus Drive,
University of Saskatchewan,
Saskatoon, Saskatchewan, Canada,
S7N 5A8

ABSTRACT

In durum wheat (*Triticum turgidum* L. var *durum*), grain protein concentration (GPC) and gluten quality are among the important factors influencing pasta-making quality. Semolina with high protein content produces pasta with increased tolerance to overcooking and greater cooked firmness. However, genetic improvement of GPC is difficult largely because of its negative correlation with grain yield, and a strong genotype x environment interaction. Therefore, identification of quantitative trait loci (QTL) for high GPC and the associated markers is a priority to enhance selection efficiency in breeding durum wheat for elevated GPC. At a physiological level, GPC is influenced by several factors including nitrogen remobilization from vegetative organs and direct post-anthesis nitrogen uptake (NUP) from the soil. Understanding the relationship between elevated GPC and nitrogen remobilization, and post-anthesis NUP will enable durum wheat breeders to develop varieties that not only produce high yield and high GPC, but also exhibit better nitrogen use efficiency. The objectives of this study were: (1) to identify and validate QTL for elevated GPC in two durum wheat populations; and (2) to determine if elevated GPC is due to more efficient nitrogen remobilization and/or greater post-anthesis NUP. A genetic map was constructed with SSR and DArT® markers in a doubled haploid population from the cross Strongfield x DT695, and GPC data were collected in replicated trials in six Canadian environments from 2002 to 2005. Two stable QTL for high GPC, *QGpc.usw-B3* on chromosome 2B and *QGpc.usw-A3* on 7A, were identified. Strongfield, the high GPC parent, contributed the alleles for elevated GPC at both QTL. These two QTL were not associated with variation in grain weight (seed size) or grain yield. *QGpc.usw-A3* was validated in a second Strongfield-derived population as that QTL was significant in all six testing environments. Averaged over five locations, selection for *QGpc.usw-A3* resulted in a +0.4% to +1.0% increase in GPC, with only small effects on yield in most environments. A physiological study of grain protein accumulation revealed that regardless of the growing condition, nitrogen remobilization was the major contributor for grain nitrogen in durum genotypes evaluated, accounting for an average of 84.3% of total GPC. This study confirmed that introgression of *Gpc-B1* into Langdon resulted in

increased GPC, and this GPC increase was due to higher N remobilization. Strongfield expressed greater N remobilization than DT695 and the semi-dwarf cultivar Commander, but N remobilization was not the determining factor for Strongfield's elevated GPC. Strongfield expressed greater post-anthesis NUP than DT695. Similarly, a selection of six high-GPC doubled haploid (DH) lines from the cross DT695 x Strongfield expressed significantly greater post-anthesis NUP than six low-GPC DH selections, supporting the hypothesis that elevated GPC in Strongfield is derived from greater post-anthesis NUP. All six high-GPC DH selections carried the Strongfield allele at *QGpc.usw-A3,* suggesting this QTL maybe associated with post-anthesis NUP.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor Dr. Curtis Pozniak (Durum wheat breeder, Crop Development Center) for his continuing support and guidance throughout my Ph.D program. His teaching on quality research and breeding program will be the upmost invaluable experience for my future career.

I am also grateful to Dr. Ravi Chibbar (Canada Research Chair in Crop Quality, Department of Plant Sciences) for serving as my co-supervisor, as well as committee members Dr. Bruce Coulman (Head, Department of Plant Sciences), Dr. Brian Rossnagel (Barley and Oat Breeder, Crop Development Center), Dr. Rosalind Bueckert (Crop Physiologist, Department of Plant Sciences), Dr. Fran Walley (Head, Department of Soil Sciences) and Dr. Dean Spaner (External examiner, University of Alberta) for their comments and suggestions. Germplasm provided by Dr. John Clarke (Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, Swift Current) is gratefully acknowledged.

The financial assistance provided by the NSERC Canada is greatly appreciated. I am also thankful to the Technological and Professional Skill Development Project (TPSDP) 2004 – the Ministry of National Education Indonesia at Universitas Jenderal Soedirman, Purwokerto, Central Java, Indonesia for the scholarship of my Ph.D program.

My sincere thanks are also to Nizar Hirji and Charlene Vinh Tang from the durum wheat field lab for their help with field experiments, Conny Briggs from the quality lab for her technical guidance and facilities for quality analysis, and Akiko Tomita and Krista Wiebe from the wheat molecular lab for their help with molecular analysis. Finally, I am forever indebted to my wife Hardiyati, my sons Lutfan and Luthfie, and my daughter Rosana for their enduring and unselfish life-long support.

THIS THESIS IS DEDICATED TO *IN MEMORIAM* MOM AND DAD WHO HAVE TOUGHT ME ABOUT HARDWORK AND PERSISTENCE

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AACC	:	American Association of Cereal Chemists
AAFC	:	Agriculture and Agri-food Canada
AFLP	:	Amplified fragment length polymorphism
ANOVA	:	Analysis of variance
ASP	:	Asparagine
DArT	:	Diversity Array Technology
DH	:	Doubled haploid
EST	:	Expressed sequence tags
Fd-	:	Ferredoxin-
Glu	:	Glutamine
GN	:	Grain nitrogen
GxE	:	Genotype by environment
GPC	:	Grain protein concentration
GS	:	Glutamine synthetase
GOGAT	:	Glutamate-oxoglutarate amino transferase (Glutamate synthase)
GrainNR	:	Proportion of remobilized nitrogen to total grain nitrogen content
HMW-GS	:	High molecular weight Glutenin sub-unit
KWT	:	Kernel weight
LMW-GS	:	Low molecular weight Glutenin sub-unit
LS	:	Least square
MAS	:	Marker assisted selection
MQM	:	Multiple QTL model
N	:	Nitrogen
NAC	:	Nitrogen assimilation control
NAD	:	Nicotinamide adenine dinucleotide
NADH	:	reduced (plus hydrogen) form of NAD
NAD(P)H	:	reduced (plus hydrogen) form of NAD-Phosphate
NAR	:	Nitrate reductase
NHI	:	Nitrogen harvest index

NILs	:	Near isogenic lines
NIR	:	Nitrite reductase
NIRS	:	Near-infrared Reflectance Spectrophotometer
NR	:	Remobilized nitrogen
NUP	:	Nitrogen uptake
RFLP	:	Restriction fragment length polymorphism
RG	:	Regina
PAGE	:	Polyacrylamide gel electrophoresis
A-PAGE	:	Acid-Polyacrylamide gel electrophoresis
SC	:	Swift Current
SDS	:	Sodium Dodecyl Sulphate
SED	:	Standard error of difference
SEM	:	Standard error of means
SIM	:	Simple interval mapping
SNP	:	Single nucleotide polymorphism
SSR	:	Simple sequence repeats
ST	:	Saskatoon
TWT	:	Test weight
USA	:	United States of America
QTL	:	Quantitative trait loci
TPN	:	Total plant nitrogen
TPN-An	:	Total plant nitrogen at anthesis
VegN-PM	:	Vegetative nitrogen at physiological maturity
ZGS	:	Zadocks growth scale

Chapter 1

INTRODUCTION

1.1. Background

Durum wheat (*Triticum turgidum* L. var. *durum*) is the main raw material used in the manufacture of pasta products. The firmness of cooked pasta and tolerance to overcooking are the two main characterists on which cooking quality of pasta depends. These two characteristics are strongly related to grain protein concentration (GPC) (Dexter and Matsuo, 1977; Grzybowski and Donnelly, 1979). As such, genetic improvement of GPC has been the target of durum wheat breeding programs worldwide (Olmos *et al*., 2003). However, genetic improvement of GPC has been slow, largely because the lack of sufficient genetic variation within adapted germplasm (Blanco *et al*., 2006), the negative correlation with grain yield (McNeal *et al.*, 1972; Steiger *et al.*, 1996), and the strong influence of the environment on its expression (Khan *et al*., 2000). Adequate nitrogen fertilizer application rate and appropriate application timing can be used to elevate GPC (Feillet, 1988), but the continued use of fertilizers is becoming less economically appealing.

Several studies have been conducted to identify genes for improved GPC in durum wheat. A promising source of high GPC was identified in a wild population of tetraploid wheat (*T. turgidum* var. *dicoccoides*) (Acc. FA-15-3) referred to as DIC (Avivi, 1978). Through an analysis of complete sets of Langdon-DIC disomic substitution lines, Joppa and Cantrel (1990) found that the gene(s) for high GPC was present on chromosome 6B. Complete substitution of DIC-6B into the cultivar Langdon, designated as Langdon(DIC-6B), resulted in significantly higher GPC and protein yield with no significant effects on protein quality, plant height, heading date, or grain yield (Chee *et al.*, 2001). Introgression of DIC-6B into common wheat varieties also resulted in GPC increase (Mesfin *et al.*, 1999). However, DePauw *et al.* (1998) concluded that Langdon(DIC-6B) does not provide protein genes superior to those already available in Canadian durum wheat germplasm as lines carrying this chromosomal region did not achieve GPC higher than commercial durum wheat

cultivars. In addition, this chromosomal region appeared to lower test weight (TWT), a trait important in determining durum sample grade (DePauw *et al*., 1998).

Grain protein concentration is a quantitative trait controlled by a complex genetic system (Blanco *et al*., 2006). Quantitative trait loci (QTL) for GPC in both hexaploid and tetraploid wheat have been reported on at least a dozen chromosomes (Prasad *et al*., 2002; Sourdille *et al*., 2003; Blanco *et al*., 2002; Joppa *et al*., 1997; Blanco *et al*., 1996, 1998, Knox *et al*., 2004). In tetraploid wheat, the majority of QTL have been identified in *T. turgidum* L. var. *dicoccoides* (Blanco *et al*., 2002; Olmos *et al*., 2003; Gonzalez-Hernandez *et al*, 2004; Blanco *et al.*, 2006), a wild relative of durum wheat. A QTL accounting for 66% of phenotypic variation in GPC, designated *QGpc.ndsu.6Bb*, has been localized (Joppa *et al*., 1997), and mapped as a simple Mendelian locus designated as *Gpc-B1*. Map-based cloning of *Gpc-B1* revealed the gene conferring elevated GPC is a N assimilation control (NAC) transcription factor (*TtNAM-B1*) (Distelfeld *et al*. 2006) associated not only with increased GPC, but also increased zinc and iron content, leaf senescence, and enhanced N remobilization (Uauy *et al*., 2006a). However, the use of *Gpc-B1* from *T. dicoccoides* in Canadian breeding programs has been limited. Thus, identification of QTL associated with elevated GPC in local durum wheat germplasm would be useful.

At a physiological level, GPC is influenced by a number of factors including N uptake, assimilation, and remobilization from leaves and stem to the grain during grain filling. Most of the N found in protein of mature cereal grains is remobilized from senescing vegetative tissues (People and Dalling, 1988; Feller and Fischer, 1994). Therefore, in wheat, high GPC has been most associated with more efficient N remobilization from senescing tissue to grain (Kichey *et al*., 2007; Clarke, 2005; Blackman and Payne, 1987). In wheat, gene(s) affecting N remobilization have been reported on 7B (Fatta *et al.*, 2000) and 6A, 1B, 4B and 3D (Khodos *et al.*, 1987). Gene(s) localized on chromosomes 1A, 4A, 7B and 1D have been identified that control post-anthesis N uptake (NUP) in wheat (Khodos *et al.*, 1987). Deckard *et al.* (1996) also reported that higher GPC in Langdon(DIC-6B) is because of higher N remobilization to the grain. In barley (*Hordeum vulgare* L.), however, nitrogen remobilization efficiency was not correlated with higher GPC (Mickelson *et al.*, 2003).

At present, studies on N remobilization and post-anthesis NUP in relation to variable expression of GPC in durum wheat germplasm are lacking. Such studies are important to elucidate the physiological parameters and distinct genes contributing to elevated GPC (Jukanti and Fisher, 2008). This knowledge will aid durum breeders to develop varieties that are not only high grain yield and GPC, but that express better N use efficiency.

1.2. Research hypothesis

Improvement of GPC in Canadian durum wheat breeding program has been made through the use of local germplasm, and evidence suggests that further increases in yield and protein concentration are still possible (Clarke, 2005). Strongfield, a Canadian durum wheat variety released in 2003, does not have *Gpc-B1 gene*, but has consistently displayed high levels of GPC coupled with high yield in Canadian environments, and has been used extensively in Canadian durum wheat crossing programs (Clarke *et al*., 2005). High GPC is presumed to be the result of pyramiding of genes related to post-anthesis N uptake and remobilization within the plant (Clarke, 2005). Therefore, this research was designed to test hypotheses that:

1. novel QTL for elevated GPC exist in local durum wheat germplasm;
2. elevated GPC is associated with better N remobilization and/or higher post-anthesis N uptake.

1.3. Research Objectives

The research objectives were:

1. to identify QTL and markers associated with elevated GPC in durum wheat;
2. to determine if elevated GPC is due to more efficient N remobilization and/or greater post-anthesis N uptake.

Chapter 2

LITERATURE REVIEW

2.1. Durum wheat

Durum wheat (*T. turgidum* L. var. *durum*) is an allotetraploid (2n = 4x = 28) with seven homoeologous chromosome pairs (AABB) (Nachit *et al*., 2001). The A genome in durum wheat originated from the diploid wild wheat (einkorn) (*T. uratu* Tum.), whereas the B genome is thought to derive from *Aegilops speltoides* Tausch (Gooding and Davies, 1997). Durum wheat is milled to semolina which is used primarily for production of pasta products (pasta, spaghetti, and macaroni). Use in non-pasta products (leavened and unleavened bread, and bulgur) is increasing, particularly in Mediterranean regions (Elias and Manthey, 2005), but the lack of the D genome found in hexaploid wheat (*T. aestivum* L.; AABBDD) greatly reduces durum wheat baking potential (Kerber and Tipples, 1969). Historically, durum wheat has been grown in Mediterranean climates in North Africa, southern Europe, Turkey, and Syria as durum wheat production is better suited to semi-arid climates (Elias and Manthey, 2005). In North America, durum wheat is produced in the dry growing regions of western North Dakota and Montana in the USA, and southern Saskatchewan and Alberta in Canada (AAFC, 2005). Annual world production of durum wheat averages 35.9 million tonnes (AAFC, 2005), accounted for approximately 5% of the total wheat production. In Canada, durum wheat comprises of 20 to 25% of total wheat area, and produces an average of 4.5 million tonnes of grain per year. Canadian durum represents more than 60% of world durum wheat trade with 80% of production exported into high quality global pasta markets (Clarke, 2005). Breeding for pasta quality is, therefore, a primary objective in Canadian durum breeding programs.

2.2. Durum wheat end-use quality

Durum wheat quality can be broadly defined into physical and chemical quality attributes, and rheological and processing characteristics. Physical grain quality traits include test/hectoliter weight, kernel weight and proportion of vitreous kernels (Clarke

et al., 1998). Chemical grain quality traits include the content of yellow pigments, protein concentration and gluten quality. The later two traits greatly influence the rheological properties of dough.

The physical quality of grain is an important grading factor as it can influence the semolina milling potential of durum grain. Test weight is a measure of grain density, and is widely utilized as a wheat grading factor. Test weight is moderately heritable with heritability estimates ranging from 0.44 to 0.83 (Bhatt and Derera, 1975). In general, lower test weights are associated with reduced semolina milling yield, but there is no consensus on the use of test weight as a predictor of milling yield in wheat (Dexter and Edwards, 1999). Thousand-kernel weight is a measure of average kernel size. In wheat, 1000-kernel weight is under genetic control and QTL have been reported on chromosomes 3D and 4A in hexaploid wheat (McCartney *et al*., 2005). There is also a strong environmental influence with heritability estimates ranging from of 0.37 to 0.69 (Sharma and Knott, 1964; Jochum *et al*., 2001; Collaku and Harrison, 2005). Larger kernels show negative association with protein content and gluten strength (Khattak *et al*., 2005), and thus can have a pleiotropic effect on the rheological properties of semolina produced dough.

The yellow colour of semolina and pasta is an important end-use quality trait in international markets (Troccoli *et al*., 2000). 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 (Hentschel *et al*., 2002). The genetics of yellow pigment are well understood in durum wheat and the trait is highly heritable (Elouafi *et al*., 2001; Clarke *et al*., 2006). Quantitative trait loci for yellow pigment have been identified on most chromosomes, but a QTL with a large effect has been identified in several mapping populations on the distal region of the long arm of chromosome 7B (Elouafi *et al*., 2001; Pozniak *et al*., 2007; Zhang and Dubcovsky, 2008). A homeologous QTL with a similar effect has also been reported on 7AL (Singh *et al*., 2009). 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).

Grain protein concentration (GPC) and gluten quality are recognized as the most important determinants of the cooking quality of pasta products (Dexter and Matsuo,

1980; D'Egidio *et al*., 1990) as these two factors influence the rheological properties of semolina dough (Payne *et al*., 1984; Pogna *et al*., 1994). Both traits have been studied intensively (Pagnotta *et al*., 2005) and generally, high GPC is associated with reduced pasta stickiness and increased tolerance to over-cooking (Marchylo *et al*., 1998). An increase in GPC is usually associated with an increase in the amount of gluten proteins (Dexter and Dronzek, 1975a; 1975b). However, an increase in GPC does not guarantee good gluten strength (Ciaffi *et al*., 1991). High GPC is most important when high-temperature drying technology is used to improve the cooking quality, colour and nutritional quality of pasta (Mercier and Hyberg, 1995; De Stefanis *et al*., 1990), as high temperature drying of pasta is known to modify physicochemical characteristics of the gluten proteins (De Stefanis *et al*., 1990). At low temperature processing, both GPC and gluten quality have similar importance in determining pasta cooking quality (Novaro *et al*., 1993; Dexter and Matsuo, 1980).

The gluten proteins have been studied intensively to determine their structural properties and to provide a basis for manipulating and improving wheat end-use quality (Shewry *et al*., 1995). Gluten is a complex mixture of two groups of proteins, the gliadins and glutenins, associated with lipids and other components (mineral and carbohydrates). High hydrophobicity of gluten proteins prevents the penetration of water into pasta during cooking and therefore prevents swelling, surface disintegration, and pasta stickiness (Feillet, 1988). Strong gluten proteins with high elastic recoveries exhibit good cooking quality, whereas weak gluten proteins with low elastic recoveries have poorer cooking quality (Feillet, 1988). The viscoelasticity of the gluten depends on the respective quantities of gliadins and glutenins, and also on the degree of sulfur bonding between the different proteins. Pasta cooking quality is superior in cultivars with a high glutenin-gliadin ratio or a high percentage of "insoluble" proteins (Feillet, 1988).

Given the importance of GPC on end-use quality in wheat, the following sections are devoted to reviewing the main groups of grain proteins, and their relationships with end-use quality in durum wheat.

2.3. Wheat grain proteins

The ability of wheat to be processed into different products is largely determined by the grain protein concentration (Weegels *et al*., 1996) and the type of proteins present in the seed at maturity (Shewry *et al*., 1986). In wheat, grain proteins can be classified on the basis of their solubility in different solvents: albumins (soluble in water), globulins (salt), and prolamins (alcohol or dilute acid/alkali) (Shewry *et al*., 1986).

2.3.1. Albumins and globulins

The albumins and globulins constitute 10 to 22% of the total wheat grain proteins (Singh and MacRitchie, 2001). These proteins are believed to have dual roles as nutrient reserves for the germinating embryo and as inhibitors of insects and fungal pathogens prior to germination (Shewry *et al*., 1984; Buonocore *et al*., 1985, Østergaard *et al*., 2000; Garcia-Olmedo *et al*., 2002). The puroindolines are included in this group, and these proteins are known to influence grain hardness. In hexaploid wheat, kernel hardness is largely controlled by the action of two linked puroindoline a (*Pina-D1*) and puroindoline b (*Pinb-D1*) genes on the distal end of the short arm of chromosome 5D (Bhave and Morris, 2007). These genes code for two cysteine-rich puroindoline a (PINA) and b (PINB) proteins (Morris, 2002), both of which contain a tryptophan domain that interact with lipids located on the surface of starch granules (Gautier *et al*., 2000; Bhave and Morris, 2008). Soft texture is the wild type phenotype, with hard texture resulting from mutations in either *Pina-D1* or *Pinb-D1*. Since durum wheat lacks the D genome, the complete absence of *Pina-D1* and *Pinb-D1* and their coded proteins results in a very hard endosperm (Giroux *et al*., 2000). However, the two *Pin* genes do not explain all of the phenotypic variation for kernel hardness among wheat varieties or within crosses of the same textural class (Matus-Cadiz *et al*., 2008). Grain softness proteins (GSPs) are closely related to puroindolines (Gautier *et al*., 2000) and are believed to have some role in determining grain hardness. However, the grain softness protein-1 gene (*Gsp-D1*) is closely linked to *Pina* and *Pinb* (Tranquilli *et al*., 1999; Turnbull *et al*., 2003) making it difficult to ascertain its direct role on grain kernel hardness (Tranquilli *et al*., 2002). Recently, an additional PIN gene has been identified, but its role in grain hardness has yet to be determined (Morris, personal

communication).

It is generally accepted that the albumins and globulins do not influence the rheological properties of wheat dough *per se* (Damodaran, 1996) but do influence the processing properties of wheat products. During milling, hard textured wheats produce coarser flour with higher levels of starch damage. Damaged starch granules absorb more water, making wheat with hard kernels better suited for bread and other yeast-leavened foods. In contrast, soft endosperm wheat fractures easily with little starch damage, making it more suitable for preparing cookies, cakes, and pastries. Durum is milled into coarse semolina which has high water absorption capacity and is favoured for production of pasta products.

2.3.2. Prolamins

The prolamins represent the largest portion of the wheat storage proteins and are classified into two groups, the gliadins and glutenins, according to their solubility in aqueous/alcohol solutions (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 80-85% of the total grain protein, and confer elasticity and extensibility properties that are essential for the rheological functionality of wheat doughs (Shewry *et al*., 1995; Feillet, 1988; Shewry and Halford, 2002).

The glutenins are polymeric proteins resulting from intermolecular disulfide bonds (Schofield, 1994; Gianibelli *et al*., 2001). After treatment with a reducing agent, the glutenins can be subdivided into high molecular sub-unit proteins (HMW-GS; 100-140 kDa) and low molecular sub-unit proteins (LMW-GS; 30-75 kDa) (Gianibelli *et al*., 2001). High polymorphism for both HMW-GS and LMW-GS coding genes have been reported in both bread and durum wheat (Payne *et al*., 1983; Gupta and Shepherd, 1990a, 1990b; Branlard *et al*., 1989).

The HMW-GS, only represents 5-10% of the total grain protein quantity, but they are major determinants of gluten elasticity (Gianibelli *et al*., 2001; Wieser, 2007). In wheat, the HMW-GS are encoded by genes present at the *Glu-1* loci located on the

long arm of homoeologous group 1 chromosomes (Lafiandra *et al*., 1984; Nachit *et al*., 2001; Elouafi and Nachit, 2004). Each locus consists of two tightly linked genes coding for two distinct HMW-GS: the HMW-GS *x*-type and HMW-GS *y*-type (Gianibelli *et al*., 2001). The y-type gene coded at the *Glu-A1* locus is not functional in the majority of tetraploid and hexaploid wheat cultivars, whereas x-type gene expression at the same locus and the y-type at the *Glu-B1* locus is variable among cultivars. This leads to variation in the number of subunits from three to five in bread wheat and from two to three in durum wheat (Lafiandra *et al*., 1984). Most durum wheats, including all registered Canadian durum varieties, carry null alleles at the *Glu-A1* loci (Du Cros, 1987; Branlard *et al*., 1989; Rao, 2008). In contrast, a high level of polymorphism has been observed at *Glu-A1* in *T. durum* var. *dicoccoides* (Nachit *et al*., 1995; Elouafi and Nachit, 2004), and these have been suggested as a potential source of allelic variation to improve gluten quality in durum wheat (Ciaffi *et al*., 2008).

Several studies have examined the relationship between HMW-GS variation and pasta quality. Good pasta is associated with the HMW-GS allelic combinations of *1Bx13+1By16*, *1Bx7+1By8*, or *1Bx6+1By8* (Motalebi *et al*., 2007). In contrast, poor pasta quality is observed in cultivars carrying the *1Bx20* (Pogna *et al*., 1990; Gianibelli *et al*., 2001). Older Canadian durum wheat cultivars including Stewart-63 and Arcola possess the *1Bx7+1By8* HMW-GS, similar to high quality bread wheat cultivars, but recent Canadian durum wheat cultivars like Strongfield (Clarke *et al*., 2005), Commander (Clarke *et al*., 2006), AC Navigator (Clarke *et al*., 2001), and CDC Verona (Pozniak *et al*., 2009) possess *1Bx6+1By8* (Rao, 2008).

The LMW-GS constitute the majority of wheat storage proteins and represent approximately 40% of the total wheat gluten fraction (Payne *et al.*, 1987; Gupta *et al*., 1989; Ciaffi *et al*., 1999). The LMW-GS are encoded by genes at the *Glu-A3, Glu-B3* and *Glu-D3* loci on the short arms of the homeologous group 1 chromosomes (Gupta and Shepherd, 1990a, 1990b). The LMW-GS encoded at the *Glu-B3 locus* are most important 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 among some durum wheat genotypes where the presence of LMW-2 glutenin subunits confers stronger gluten than cultivars possessing LMW-1 (D'Ovidio, 1993; Vazquez *et*

al., 1996). Most recent durum wheat cultivars express the LMW-2 pattern, but considerable variation in gluten strength is still present (Rao, 2008).

Based on acidic polyacrylamide gel electrophoresis (A-PAGE), gliadins can be classified into: α, β, γ and ω gliadins, respectively (Bushuk and Zillman, 1978). 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 residues (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 have been postulated to interact with other proteins through disulphide interchanging, and through hydrophobic and hydrogen bonding (Bietz 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. Genes encoding gliadin components are located on chromosomes of homoeologous groups 1 and 6 (Joppa *et al.,* 1983). Genes coding for α and β gliadins are clustered at homeologous loci *Gli-A2* and *Gli-B2* on the short arms of the group 6 chromosomes, whereas the genes coding for the γ and ω gliadins are clustered at *Gli-A1* and *Gli-B1* on the short arms of the chromosomes 1A and 1B (Troccoli *et al*., 2000). Allelic polymorphism is higher for gliadins than for glutenins (Metakovsky and Branlard, 1998). The *Gli-B1* loci that encode γ- and ω-gliadins are tightly linked to the *Glu-B3* locus (Brown and Flavell, 1981). Several studies have examined the relationship between gliadin proteins and dough rheological properties in wheat (Wrigley *et al*., 1981; Pogna *et al*., 1982; Dachkevitch *et al*., 1993). Durum wheat varieties that possess the γ-45 gliadin fraction have high intrinsic cooking quality, whereas those possessing the γ-42 gliadin have poorer cooking quality (Damidaux *et al.,* 1978; Gianibelli *et al*., 2001). In durum, γ-45 is linked with ω-35 and a group of LMW-2 glutenin subunits, while γ-42 is associated with the ω components 33, 35 and 38 and LMW-1 glutenin subunits (Payne *et al.,* 1984). At present, most durum wheat breeding programs have fixed the LMW-2/γ-45/ω-gliadin 35 loci because of their positive effects on pasta quality. However, large

differences in pasta quality are still evident in lines carrying these proteins, suggesting other factors also influence pasta quality (Galterio *et al*., 1993).

2.4. Physiology and genetics of GPC

2.4.1. Physiology of GPC

Nitrogen (N) is one of the building blocks of amino acids, proteins, and nucleic acids; therefore, it is one of the major limiting factors for plant growth and development (Barneix *et al*., 2007). The supply of N to the developing grain originates both from the remobilization of N stored temporarily in vegetative plant parts and from post-anthesis N uptake (NUP) transferred directly to kernels (Figure 2.1; Kichey *et al.*, 2007; Dupont and Altenbach, 2003). Each of these processes will be discussed in relation to GPC in the followings sections.

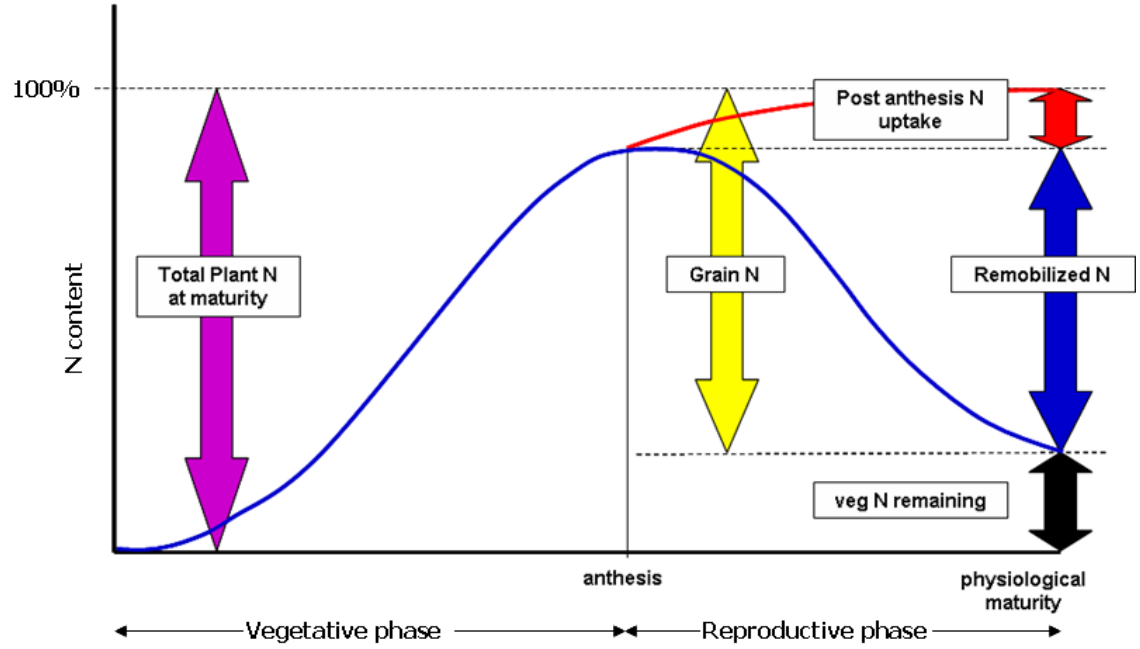

Figure 2.1. Grain N accumulation in relations to N remobilization and post-anthesis N uptake. Blue cruve line represents the graph of N content in the vegetative organs that reach its maximum at anthesis, followed a decline due to N remobilization to the grain. Red curve line represents the graph of post-anthesis N uptake.

2.4.1.1. Nitrogen uptake and assimilation

In cereals, N for assimilation is derived from soil organic matter. Soil inorganic N pool, and N-fertilizer application. In the absence of fertilizer N, the major source of

fixed nitrogen in the plant is from soil organic matter undergoing decomposition. During soil organic matter decomposition, excess ammonium (NH_4^+) not utilized by microbes is released, and subsequently oxidized by autotrophic organisms to nitrite (NO_2^-) and then nitrate (NO_3^-), the key source of N supply for cereal plants (Haynes and Goh, 1978). Therefore, the availability of N depends on the amount of organic matter present, and on the presence of microbial populations and conditions favoring their activity. In the main durum growing regions of western Canada, organic matter content is relatively low and soils are usually deficient in available N (Campbell *et al*., 1990). As such, additional N fertilizer is required to achieve adequate yields and elevated GPC (Grant and Flaten, 1998).

During vegetative growth, plants absorb N from the soil to meet the needs of structural growth. Plants may utilize both nitrate and ammonium ions, but under normal aerated soil conditions, nitrate is the main source of N (Barker and Bryson, 2007). In plants, soil N uptake is controlled by genes encoding nitrate transporters. Some high-affinity nitrate transporter genes have been cloned from several plants, including: barley (*Hordeum vulgare L.*) (Trueman *et al*., 1996; Vidmars *et al*., 2000); *Arabidopsis thaliana* (*AtNRT1.1*; Zhuo *et al*., 1999; Filleur and Daniel-Vedele, 1999); *Nicotiana plumbaginifolia* (*NpNRT2.1*; Quesada *et al*., 1997); and soybean (*Glycine max* L.) (Amarasinghe *et al*., 1998). There is evidence that several genes encoding nitrate transporters are present in cereals. In barley, as many as ten putative members of *Nrt2* gene families have been reported (Trueman *et al*., 1996).

Nitrogen assimilation in plants is complex (Figure 2.2), and involves three major gene families, those coding for nitrate reductase (NAR), nitrite reductase (NIR), and glutamine synthetase / glutamate synthase (glutamate-oxoglutarate amino transferase) (GS/GOGAT) (Miflin and Habash, 2002; Good *et al*., 2004; Boisson *et al*., 2005). Nitrate is readily mobile in plants and can be stored in vacuoles, but must be reduced to ammonium to be used in the synthesis of proteins and other organics compounds (Barker and Bryson, 2007). During early nitrogen assimilation, nitrates are first reduced to nitrites by nitrate reductase (EC 1.7.1.1, NADH-NAR; EC 1.7.1.20, NAD(P)H-NAR) in the cytoplasm. Nitrites are then translocated to the chloroplasts, where they are further reduced by Ferredoxin-dependent nitrite reductase (Fd-NIR; EC 1.7.7.1) to

ammonium (Good *et al*., 2004). Once in the form of ammonium, GOGAT works conjointly with GS in the first step of ammonia assimilation and catalyses the reductant-dependent conversion of glutamine and 2-oxaloglutarate to two molecules of glutamate. GOGAT exists as two distinct isoforms, ferredoxin dependent (Fd-GOGAT; EC 1.4.7.1) and NADH dependent (NADH-GOGAT; EC 1.4.1.14) (Boisson *et al*., 2005). Fd-GOGAT is the predominant form and plays an important role in leaf photorespiratory ammonium assimilation. In contrast, NADH-GOGAT is most active in non-photosynthetic tissue (Good *et al*., 2004). Although its exact physiological function remains unclear, glutamate dehydrogenase (EC 1.4.1.14; NAD-GDH) has also been associated with inorganic nitrogen assimilation (Barneix *et al*., 2007).

Several of the genes coding for enzymes involved in nitrogen assimilation have been sequenced. Nitrate reductase is a homodimeric enzyme, and genes encoding NAR have been sequenced in several plants, including *Arabidopsis* (Wilkinson and Crawford, 1993; Cheng *et al*., 1988), maize (*Zea mays* L.) (Gowri and Campbell, 1989), and barley (Schnorr *et al*., 1991). In durum, expression of NAR is induced by nitrates, but light is required for induction and expression follows a circadian rhythm (Carillo *et al*., 2005), similar to other plants (Deng *et al*., 1990; Duke and Duke, 1984). The gene for Fd-dependent GOGAT has been characterized in maize (Sakakibara *et al*., 1991) and barley (Avila, 1993), while two genes have been identified in Arabidopsis (Suzuki and Rothstein, 1997; Coschigano, 1998) and *N. plumbaginifolia* (Ficarelli *et al*., 1999). In wheat, although no sequence data has been reported, NADH-NAR genes have been physically mapped to chromosomes 6A, 6D and 7A and 7D (Kilian *et al*., 1992). Boisson *et al*. (2005) genetically mapped Fd-GOGAT on chromosome 2DS.

Following assimilation, organic nitrogen compounds are transported between organelles, from cell to cell and over long distances in support of plant metabolism and development (Rentsch *et al*., 2007). Amino acids (including amides) represent the principal transport form for organic N, but some plant species (warm season legumes fixing nitrogen) use ureides for long distance N transport (Rentsch *et al*., 2007). Nitrogen is shuttled between compounds via the activity of transaminases and glutamine-amide transferases, but most is released as ammonium or an amino group and re-assimilated via the GS pathway (Miflin and Habash, 2002).

The largest fraction of assimilated N is used for protein synthesis, while smaller fractions are present in nucleic acids and in primary and secondary metabolites (Peoples and Dalling, 1988). In wheat leaves, 75% of the N is present in the chloroplast, with stromal (especially ribulose-1,5-bisphosphate carboxylase/oxygenase [Rubisco]) and thylakoidal proteins representing the major fractions of chloroplast N (Peoples and Dalling, 1988; Hörtensteiner and Feller, 2002).

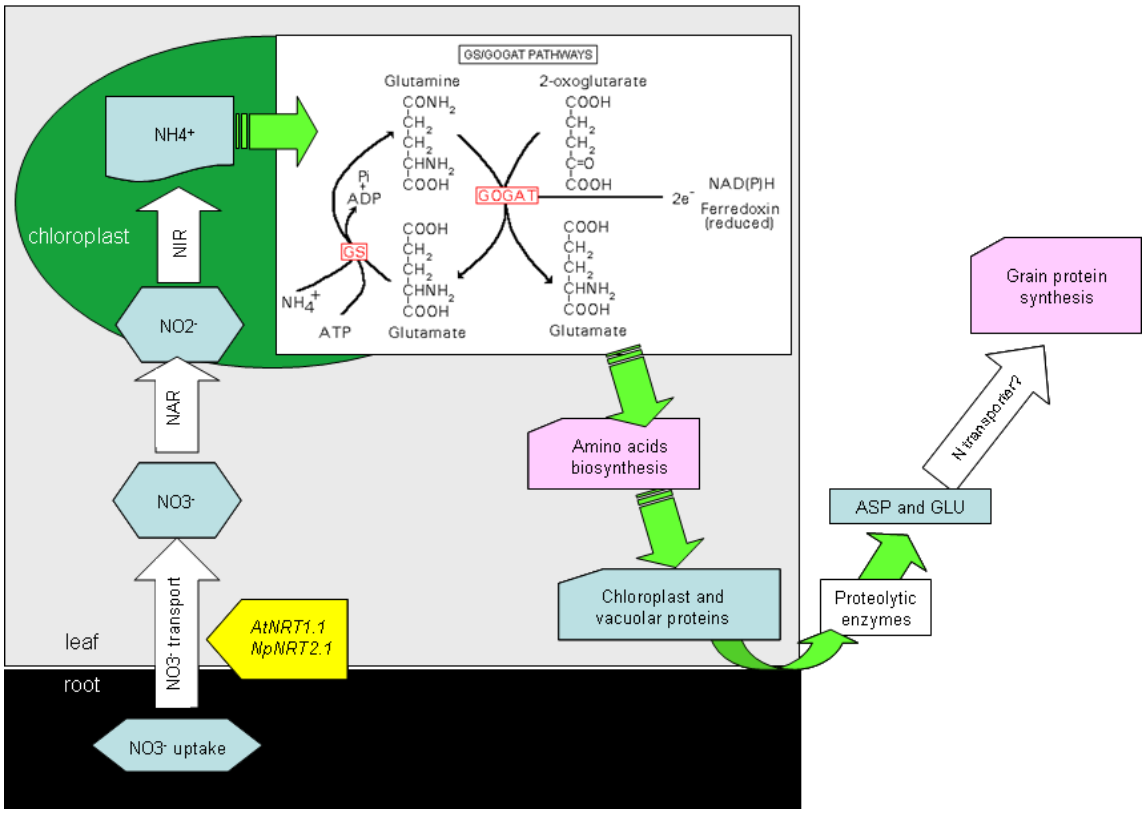

Figure 2.2. A simplified pathway of nitrogen assimilation in plants that includes nitrogen uptake, assimilation, protein synthesis, protein degradation, phloem transport, and protein re-synthesis in the grains. The *AtNRT1.1* and *NpNRT2.1* are genes known to control high-affinity N transport during N uptake. ASP = asparagines, GLU = glutamine. The GS/GOGAT pathway in white box is adapted from http://www.hort.purdue.edu/rhodcv/hort640c/ammonia/am00002.htm

2.4.1.2. Nitrogen remobilization

N remobilization is a necessary physiological process to meet the requirements for grain yield and/or protein content (Sinclair *et al*., 2000) as the amount of N absorbed

during grain development is less than the demand for N in developing grains. Senescence represents the final stage of leaf development and is characterized by the transition from nutrient assimilation to nutrient remobilization (Feller and Fischer, 1994). A large portion of N is translocated from senescing vegetative tissues to the developing grains for protein synthesis (Mae, 1997; Zhu *et al*., 2007).

The hydrolysis of proteins to free amino acids for subsequent remobilization is required for N transport and depends on the action of endo- and exopeptidases (Brouquisse *et al*., 2001; Callis, 1995; Kondrat'-ev and Kamalova, 1983) most of which are upregulated in senescing vegetative tissues (Hörtensteiner and Feller, 2002; Gepstein, 2004). Three major protein degradation pathways are recognized in plants: the ubiquitin-dependent, chloroplast degradation, and vacuolar degradation pathways (Vierstra, 1996). The ubiquitin-dependent pathway is responsible largely for the degradation of short-lived and regulatory proteins in the cytoplasm and in the nuclei of eukaryotic cells (Smalle and Vierstra, 2004). Chloroplast degrading proteases target degradation of stromal proteins, including Rubisco (Hörtensteiner and Feller, 2002). Vacuolar proteolytic enzymes (serine-, cysteine-, aspartic acid-, metallo-, and carboxy-peptidases) are presumed to be active in the degradation of peptides released from the chloroplast (Callis, 1995; Distefano *et al.*, 1999; Brouquisse *et al.,* 2001). Cytosolic aminopeptidases and oligopeptidases may also cleave peptides released from plastids into free amino acids.

In plants, the amino acids glutamine and/or asparagine are preferred for long-range N transport and storage. In barley, glutamine accounts for approximately 50% of the total amino acids, while asparagine accounts for roughly 20% (Winter *et al*., 1992). In contrast, in wheat, glutamine and asparagines account for 30% and 20% of the total amino acids, respectively with these proportions changing as the plant ages (Peeters and Van Laere, 1994; Caputo and Barneix, 1997). Glutamine and asparagine are important both in terms of the role they play in metabolism (Ireland and Lea, 1999) and because of their roles in long-distance transport of reduced N in the phloem (Bush, 1999). Asparagine is an important N transport amino acid because it has a high N to carbon ratio, and is relatively inert compared to other N-transporting amino acids (Lea and Miflin, 1980; Sieciechowicz *et al*., 1988; Lea *et al.*, 1990).

The amino acids derived from protein catabolism may be transported to some tissues via the phloem with or without prior modification (e.g. production of amides from other amino acids) (Hörtensteiner and Feller, 2002). The mechanisms for exporting amino acids from the leaf cells and for loading into the phloem for N transport are still not known. However, there are some evidences that a similar mechanism for phloem loading of sucrose (probably driven by a proton symport) is used for amino acids and involves transport against a concentration gradient (Lalonde *et al*., 2004).

2.4.1.3. Synthesis of grain protein

During the early stages of grain development, the albumins and globulins, are synthesized first and coincide with the early development of embryo and aleurone layer (Simmonds, 1978). Throughout grain development, the proportion of albumins declines, while the proportion of globulins remain essentially constant (Feillet, 1988). Storage proteins first appear in the developing endosperm approx. 10 days after anthesis and are synthesized continuously until the kernel has reached physiological maturity (Mitra and Bhatia, 1973). In the grains, most proteins are deposited within the cell structure surrounding the starch granules, with smaller amounts found as fibril proteins or deposited as a protein mass (Feillet, 1988).

The rate of protein synthesis is initially high, with approximately 50% of the total storage proteins synthesized in the first 20 days after anthesis (Simmonds, 1978). Fourteen days after anthesis, nearly 25% of total grain N is in the gliadin fractions, In contrast, the percentage of glutenins increases 25 days after anthesis (Dexter and Dronzek, 1975b; Ng *et al*., 1991).

2.4.1.4 Relationships among nitrogen uptake, remobilization and GPC

In cereals, the incorporation of N into grain proteins is most associated with post-anthesis NUP and remobilization of N previously acquired in vegetative tissues prior to anthesis (Dalling, 1985; Cox *et al*., 1986; Weiland and Ta, 1992; Feller and Fischer, 1994; Dupont and Altenbach, 2003). Elevated GPC is correlated with greater plant N concentration at anthesis in rice (Ntanos and Koutroubas, 2002), and has been shown to correlate with free amino acid concentrations in the flag leaf during grain

filling in wheat (Millet *et al*., 1992; Barneix and Guitman, 1993). Uptake of nitrate and ammonia from the soil after flowering also correlates with GPC (Blackman and Payne, 1987), especially when soil N and water are adequate (Egle *et al*., 2007). In winter wheat, N remobilization and post-anthesis NUP contributes largely to grain yield and GPC; and NAR activity was highly correlated to post-anthesis NUP and GPC (Kichey *et al*., 2007). In contrast, an earlier study in barley found that post-anthesis NUP in high-GPC cultivars did not accumulate more N after heading than low-GPC cultivars (Bulman and Smith, 1994).

The relative importance of N remobilization and post-anthesis NUP to GPC is environmentally dependent, varies among genotypes, and is a function of the amount of available N for uptake, water availability, and plant health (Kichey *et al.,* 2007; Arduini *et al*., 2006). In maize, post-anthesis NUP and leaf N remobilization contribute almost equally to GPC (Hirel *et al*., 2001)**.** Indeed, Reed *et al*., (1980) reported that during maize grain development, grain protein accumulation was closely related to leaf protease activities and leaf protein degradation for subsequent translocation. In barley, pre-anthesis and post-anthesis NUP were equal contributors to grain N, especially when plants were cultivated under N-deficient conditions (Egle *et al*., 2007). In wheat, post-anthesis NUP is a greater contributor to GPC than N remobilization when adequate N and water are not limiting (Baresel *et al*., 2008), but under stress conditions, the majority of grain N is derived from N remobilization of senescing vegetative tissues (Austin *et al*, 1977; Cox *et al.*, 1985a; 1986; Papakosta and Garianas, 1991; Tahir and Nakata, 2005). In wheat, genotypic variation for N remobilization exists (Kichey *et al*., 2007; Cox *et al*., 1985b; Van Sanford and MacKown, 1987); and hexaploid wheat cultivars more efficient at translocating N from vegetative tissue to grains generally possess higher GPC (Wang *et al*., 2003). In durum, elevated GPC in the introgression line Langdon(DIC-6B) was reported to be due to better N remobilization (Deckard *et al*., 1996; Kade *et al*., 2005).

2.4.2. Genetics of GPC

2.4.2.1. Genetic variance and heritability of GPC

Grain protein concentration is controlled by a complex genetic system and

influenced by environmental factors and management practices (nitrogen and water availability, temperature and light intensity) (Blanco *et al*., 2006; Pagnotta *et al*. 2005). Genetic variation for GPC is small compared with variation due to growing environments (Khan *et al*., 2000; Galova *et al*., 1999); and heritability estimates are generally low (Clarke, 2005; Khan *et al*., 2000; Galova *et al*., 1999). Legge *et al.* (1991) reported heritability of 0.20 to 0.57 using F_3-F_5 correlations in three durum crosses. At more inbred generations of F_6-F_9, Vallega (1985) observed heritabilities of 0.38 to 0.67. Blanco *et al*. (2002) found heritabilites ranging from 0.54 to 0.78 for a set of recombinant substitution lines in a tetraploid wheat cross grown in several environments. In the later study, it is likely that higher heritability estimates were observed because only a single chromosome differed between the substitution lines.

Several environmental factors can influence expression of GPC. Elevated temperatures during durum wheat grain ripening favour the deposition of total and acetic acid insoluble proteins, while the accumulation of soluble fractions is not modified by heat stress (De Stefanis *et al*., 1998). However, grain protein accumulation is less temperature-sensitive than starch deposition, and high temperatures after anthesis reduce seed size and increases GPC (Campbell *et al*., 1981). Rapid chlorophyll degradation in the isogenic lines carrying *Gpc-B1* were also associated with a shorter grain filling period and elevated GPC (Uauy *et al*., 2006b). Elevated GPC in wheat cultivars experiencing post-anthesis drought and attack by foliar diseases late in grain fill might also be related to shortening grain filling period and sensitivity of starch deposition to adverse environmental conditions (Dimmock and Gooding, 2002).

The range in GPC of current Canadian durum wheat cultivars is small, a result of continued selection by breeders to achieve high GPC in new cultivars. When compared in common field trials, Commander (Clarke *et al*., 2006) and AC Navigator (Clarke *et al*., 2001) have approx 1.0% less protein than the highest GPC cultivar Strongfield (Clarke *et al*., 2005) and CDC Verona (Pozniak *et al*., 2009). In contrast, large variation for GPC exists in collections of emmer wheat (*T. turgidum* L. var. *dicoccoides*), the wild relative of cultivated durum wheat. Accessions of *T. dicoccoides* with grain protein concentrations of 16 to 27% have been reported (Ciaffi *et al*., 2008) and represent a useful source of genetic variability for GPC. Introgression of

chromosome 6BS of wild emmer wheat (*T. turgidum* L. var. *dicoccoides*) (Avivi, 1978; Joppa and Cantrel, 1990; Chee *et al*., 2001) in durum resulted in a 1.5% increase in GPC with little effect on yield and maturity (Chee *et al*., 2001). Chromosome 1AS and 1BS, and the homoeologous group 5 and 7 chromosomes of *T. turgidum* L. var. *dicoccoides* have also been identified to carry genes associated with GPC (Levy and Feldman, 1989). Substitution of individual chromosomes 6B, 2A, 3A and 6A from *T. turgidum* L. var. *dicoccoides* also resulted in elevated GPC (Joppa and Cantrell, 1990).

2.4.2.2. Quantitative trait loci for GPC, N remobilization and post-anthesis N uptake

Quantitative trait loci (QTL) are genomic regions that contain gene(s) associated with variation in quantitative expression of a trait (Collard *et al*., 2005). Given the importance of GPC for end-use and nutritional quality, many studies have been conducted to identify QTL for GPC. In hexaploid wheat, QTL for GPC have been mapped on at least a dozen chromosomes (summarized in Table 2.1). Prasad *et al*. (2002) identified 13 QTL for GPC distributed on eight different chromosomes with phenotypic variation (R^2) ranging from 2.9 to 32.4%. Sourdille *et al*. (2003) identified a QTL for GPC on chromosome arm 6AS (R^2 = 17.1%) in population derived from the cross Courtot x Chinese Spring. In malting barley, low protein concentration is desirable (Emebiri *et al*., 2003), and QTL for GPC have been localized on chromosomes 1H, 2H, 5H, 6H and 7H (Table 2.1; Bezant *et al*., 1997). See *et al*. (2002) reported a major QTL for GPC near marker *hvm74* on chromosome 6HS. In rice, major QTL for GPC have been mapped to a 30 cM interval on chromosome 5 (Hu *et al.*, 2004).

In durum wheat, QTL for GPC have been reported on nearly all chromosomes, but most of these QTL have been identified from wild emmer (*T. durum* var. *dicoccoides*) (Table 2.1; Joppa *et al*., 1997; Blanco *et al*., 1996, 1998, Knox *et al*., 2004). The phenotypic variation explained by individual QTL for GPC is, on average, only 25% (Joppa *et al*., 1997; Blanco *et al*., 2002; Prasad *et al*., 2002; Sourdille *et al*., 2003). QTL for protein-associated quality traits have also been reported in tetraploid and hexaploid wheat populations which include QTL for gluten strength on 2A (Knox *et al*., 2004), and QTL for SDS sedimentation volume (a measure of gluten protein

Table 2.1. Reported QTL for GPC, protein quality characteristics [updated from Charmet and Groos (2002)].

Cross	Number of environments	QTL	R^2	References
Barley: Blenheim x Kim	1	1HS, 1HL, 2HL,5HS, 6H, 7HS, 7HL	not available	Bezant *et al*., 1997
Langdon (durum) x 6B (*dicoccoides*)	-	6B	66%	Joppa *et al*., 1997
PH132 x WH711, PH133 x WH711, PH132 x HD2329	5	2AS, 2DL, 3DS, 4AL, 6BS, 7AS	8.2-32.4%	Prasad *et al*., 1999, 2003
Messapia (durum) x *T. dicoccoides*	6	4AL, 4BS, 5AL, 6AS, 6BL, 7AL, 7BS	49-56%	Blanco *et al*., 1996, 1998
Durum: Kyle2*/Biodur	-	2A	not available	Knox *et al*., 2004
Messapia (durum) x *T. dicoccoides*	6	1AL (*XgluA1*), 1BS (*XgliB1*/*gluB3*), 3AS, 3BL, 5AL, 6AL, 7BS	36-64%	Blanco *et al*., 1996, 1998
Forna (*T. aestivum*) x Oberkulmer (spelt)	3	6 QTL, one major on 5A (25%)	49%	Zanetti *et al*., 1999, 2001
Forna (*T. aestivum*) x Oberkulmer (spelt)	3	1AS (*XgluA3*), 2A, 5A common with GPC	45%	Zanetti *et al*., 1999, 2001
Bread wheat: Courtot x CS	3	1BL, 6AS	23%	Perretant *et al*., 2000
Bread wheat: Renan x Recital	6	2A, 3A, 4D, 7D	12-30%	Groos *et al*., 2003
Bread wheat: Avalon x Hobit	2	2B, 6B, 7A	10.3-18.2%	Turner *et al*., 2004

strength) on 1AL, 1BS, 3AS, 3BL, 5AL, 6AL, 7BS (Blanco *et al*., 1996, 1998) and 1AS, 2A, 5A (Zanetti *et al*., 1999, 2001) (Table 2.1).

High GPC was associated with a single QTL (designated as *QGpc.ndsu.6B*) on chromosome 6BS in Langdon(DIC-6B), that accounted for 66% of phenotypic variation (Olmos *et al*., 2003). This QTL was mapped as a simple Mendelian locus (*Gpc-B1*), and map-based cloning revealed a NAC transcription factor (*TtNAM-B1*) (Distelfeld *et al*., 2006) that was associated not only with increased GPC, but also increased zinc, and iron content (Uauy *et al*., 2006a). This gene is also associated with leaf senescence and enhance N remobilization (Uauy *et al*., 2006a), indicative of a strong association between high GPC and N remobilization in lines carrying this gene. The GPC QTL on chromosome 6HS of malting barley is orthologous to the *Gpc-B1* (Distelfeld *et al*., 2008).

Chromosomal regions for genes controlling N remobilization and post-anthesis NUP have been identified in hexaploid wheat (Fatta *et al*., 2000; Khodos *et al*., 1987), but only QTL for N remobilization have been identified in barley (Mickelson *et al*., 2003). These QTL were found to overlap with QTL for N metabolism and localized on chromosomes 3, 4, 5, and 6 (Mickelson *et al*., 2003). In hexaploid wheat, genes for N remobilization at high N supplies were detected on the short arm of chromosome 7BS (Fatta *et al*., 2000). Khodos *et al*., (1987) reported genes on chromosomes 1B, 3D, 4B and 6A affect reutilization of N, while genes on chromosomes 1A, 4A and 1D had the greatest effect on post-anthesis NUP.

2.5. Genetic improvement of GPC

2.5.1. Association of GPC with important agronomic traits

Genetic improvement of GPC has been a major target of durum wheat breeding programs (Olmos *et al*., 2003). However, yield and GPC are negatively correlated so that simultaneous improvement of both traits is difficult (Blanco *et al*., 2006; Groos *et al*., 2003; Cox *et al*., 1985b). Such a relationship could be the result of dilution of N compounds by increasing carbohydrate deposition during grain development (Cox *et al*., 1986; Jenner *et al*., 1991), or due to pleiotropic gene effects (Blanco *et al*., 2002). Competition for transport of proteins and sugars to the grain has also been

previouslydemonstrated (Fernandez-Figares *et al*., 2000). Elevated GPC is usually found under adverse growing conditions for grain formation, since the production and translocation of compounds such as carbohydrates to the grain is more sensitive than is protein accumulation (Campbell *et al*., 1981). However, studies have demonstrated the possibility of increasing yield without diminishing GPC (Loffler and Busch 1982; Stoddart and Marshall, 1990; Gooding and Davies, 1997; Rharrabti *et al*., 2001). Cox *et al*., (1986); and Beninati and Busch (1992) reported major genes conferring increased GPC without significant effects on yield, suggesting that efforts to improve GPC can be accelerated by means of identification of genes that affect GPC and direct selection of the alleles with positive effects (Olmos *et al*., 2003).

Plant height has been shown to be associated with expression of GPC in wheat. The *Rht-B1* (previously designated as *Rht1*) and *Rht-D1* (previously designated as *Rht2*) semi-dwarfing genes were introduced into commercial wheat cultivars from the Japanese variety Norin10 as part of wheat improvement programs in the USA and at CIMMYT (Ellis *et al*., 2002). Incorporation of *Rht-B1* resulted in improved lodging resistance and grain yield (Gale and Youssefian, 1985). However, lines carrying these genes produce concomitant reductions in the sub-crown internodes and coleoptile length, and leaf area of wheat seedlings (Allan *et al*., 1961; Allan, 1989; Botwright *et al*., 2001). In addition, this gene has been associated with reduced test weight in durum wheat (Clarke *et al.*, 2009).

Compared to the wild type allele (*Rht-B1a*), the dwarfing allele *Rht-B1b* contains a single base-pair change leading to a "TAG" stop codon shortly after the start of translation (Peng *et al*., 1999). This mutation results in a non-functional protein which reduces the plant's ability to respond to endogenous gibberellic acid (GA-insensitive). This allele is also thought to decrease cell wall extensibility (Keyes *et al*., 1990) and reduce epidermal cell length compared to standard height (*rht*) genotypes (Keyes *et al*., 1989; Hoogendoorn *et al*., 1990). However, lines carrying *Rht-B1* have been shown to express significantly reduced GPC in four durum crosses (McClung *et al*., 1986), and these results were confirmed in near isogenic lines with different alleles of *Rht-B1* (Pinthus and Gale, 1990). In a separate study, Blanco *et al*. (2002) reported a correlation of 0.62 between GPC and height in a tetraploid wheat population. However,

Vallega (1985) did not find any difference in GPC of tall and semi-dwarf genotypes in one durum cross, and Joppa and Walsh (1974) found no differences in semolina protein concentration for 26 isogenic semi-dwarf/tall pairs derived from 16 durum crosses. In the latter study however, none of the populations were segregating for GA-insensentive dwarfing genes. The only Canadian semi-dwarf durum varieties are Commander and AC Navigator and both carry *Rht-B1* (Pozniak, personal communiation) and express lower protein (Clarke *et al*. 2009). Lines carrying *Rht-B1* have increased seed size (Flintham *et al*., 1997), but the effects of *Rht-B1* appear to be independent of dilution effects associated with larger seeds (Pinthus and Gale, 1990). A number of alternative dwarfing genes (*Rht4* to *Rht20*) have been reported to reduce plant height in wheat but show sensitivity to exogenous GA (Gale and Youssefian, 1985; Ellis *et al*., 2004). These genes do not shorten coleoptile length or decrease seedling vigour (Rebetzke *et al*., 1999; Botwright *et al*., 2001, 2005; Ellis *et al*., 2004), but their effects on GPC are not yet known.

2.5.2. Use of wild relatives to improve GPC in wheat

It is generally recognized that the level of genetic diversity for GPC in durum wheat elite germplasm has been declining due to the recurrent use of related elite germplasm and high selection pressure applied in breeding programs, especially for grain quality traits like GPC. Recent molecular diversity studies suggest that most modern durum wheat cultivars can be traced to one or more of ten foundation cultivars (Maccaferri *et al*., 2003). Canadian cultivars can be traced to two or three founding varieties (Reimer *et al*., 2008).

Wild relatives of durum wheat have shown promise as donors of useful genes for several traits, including disease resistance, drought tolerance, and grain protein quality (Blanco *et al.*, 2006). Large variation for GPC has been reported in collections of *T. turgidum* var. *dicoccoides* (Ciafi *et al*., 2008) and the QTL for GPC have been localized on almost all chromosomes (Levy and Feldman, 1989; Joppa and Cantrell, 1990) (see section 2.3.2.1.). The *Gpc-B1* gene has been utilized in Canada (DePauw *et al*., 2005; 2007), and in US durum crossing programs (Olmos *et al*., 2003; Distelfeld *et al*., 2004; 2006) to improve GPC. However, a major drawback of using wild germplasm is that it

contains many commercially unacceptable traits (Colmer *et al*., 2006). Backcrossing has been used in durum wheat to transfer simply inherited traits but the application of backcrossing to the improvement of quantitative traits, such as GPC, has been limited mainly because of the low heritability and the difficulty of simultaneously transferring a large numbers of genes (Blanco *et al*., 2006).

2.5.3. Selection to improve GPC

Breeding for elevated GPC in wheat breeding programs has relied mostly on intercrossing of local germplasm. Depending on the breeding method employed, lines selected for agronomic merit at the F_5 or F_6 generations are usually evaluated for GPC measured using near infrared reflectance spectrophotometry (NIRS) (McCaig *et al.*, 1992; Knott, 1995). However, because of strong GxE interactions, early generation selection for GPC in durum is not effective (Clarke *et al*., 2009) and should be practiced only in multi-location trials where average performance over a range of environments can be assessed. Therefore, marker assisted selection (MAS) has been suggested as a technique to improve selection of quantitative traits like GPC (Khush, 2002).

Marker-assisted selection is a method whereby a phenotype is selected based on a marker genotype. The advantages of MAS over conventional selection include: (1) less time and resources compared to phenotypic selection for those traits requiring multi-location field trials for trait assesment; (2) identification of true genotype as there is no environmental effect involved; (3) at-seedling stage selection; (4) possibilities of gene pyramiding, or multiple genes combination; (5) a reduction of linkage drag and thus minimizing the effects of linked, deleterious genes; (6) selection of low heritability traits; and (7) testing of specific traits where phenotypic evaluation is restricted or not possible (Collard *et al*., 2005).

In wheat, several marker types are available for MAS, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and diversity array technology (DArT®) markers. In wheat, SSR markers are numerous and are the most commonly used markers (Somers *et al*., 2004). In addition, several SSR consensus maps of the wheat genome have been constructed (Somers *et al*., 2004), allowing targeted saturation of

QTL once they are identified. However, with increasing sequence efforts in wheat, use of expressed sequence tags (EST) and single nucleotide polymorphism (SNP) markers are increasing in wheat (Gao *et al*., 2004; Ganal and Röder, 2007) and durum (Pozniak *et al*., 2007; Singh *et al*., 2009).

Although numerous QTL have been identified for GPC (see Table 2.1), only a few have been used effectively to select for high GPC in wheat breeding programs (Humphreys *et al*., 1998). This is largely because a) the QTL have not been precisely localized and span 10-15 cM; b) the expression of the majority of these QTL are environmentally dependent (significant in only a few environments); c) individually, the QTL explain very little of the phenotypic variation for GPC; and d) these QTL have not been validated as a selection tool in diverse genetic backgrounds (Prasad *et al*., 1999; Dholakia *et al*., 2001; Blanco *et al*., 2002). In addition, most identified QTL for GPC are associated with a reduction in yield, the primary target of wheat breeding programs. For example, of the seven GPC QTL identified in durum wheat by Blanco *et al*. (2002), six were associated with reduced grain yield. As such, QTL mapping studies for GPC must examine the association of tagged GPC QTL with grain yield before they can be implemented successfully in wheat breeding programs. However some studies have shown the potential of selecting for GPC using marker assisted selection. Humphreys *et al*. (1998) used RFLP markers to backcross *Gpc-B1* from the wheat variety "Glupro" into two advanced breeding lines with marginal protein. The majority of lines that carried *Gpc-B1* from these crosses had 1% more GPC then their recurrent parent. DePauw *et al*. (2007) also introgressed *Gpc-B1* into solid-stemmed hexaploid wheat backgrounds using marker assisted selection. Most earlier released solid-stemmed wheat cultivars were low in GPC (DePauw *et al*., 2007), but introgression of *Gpc-B1* resulted in with GPC equivalent to hollow stemmed varieties and resulted in the release of the wheat cultivar "Lillian" (DePauw *et al*., 2005). Lillian is also resistant to stripe rust (DePauw *et al*., 2007), probably due to *Yr36,* which is tightly linked to *Gpc-B1* (Fu *et al*., 2009). Molecular transfer of *Gpc-B1* locus into American durum wheat cultivars also produced a similar response (Olmos *et al*., 2003; Distelfeld *et al*., 2004; 2006; Uauy *et al*., 2006a). However, the use of *Gpc-B1* in Canadian durum wheat breeding programs has been limited as this gene does not appear to provide any advantage to

genes already existing in durum wheat cultivars (DePauw *et al*., 1998; Kovacs *et al*., 1998). As well, introgression of this gene into Canadian elite germplasm was associated with a large reduction in test weight, an important grading factor for durum wheat in Canada. To date no commercial durum wheat cultivars grown in Canada contain this gene, but molecular assisted selection to incorporate this gene into lines carrying *Rht-B1b* is underway in Canadian durum programs (Pozniak, personal communication). It is hoped that this gene can offset the negative impact of *Rht-B1b* on expression of GPC in semi-dwarf cultivars (see section 2.4.2). However, other useful genes must be identified for incorporation into conventional height cultivars.

Chapter 3

IDENTIFICATION AND VALIDATION OF QUANTITATIVE TRAIT LOCI FOR GRAIN PROTEIN CONCENTRATION IN ADAPTED CANADIAN DURUM WHEAT POPULATIONS [*)]

3.1. Abstract

Grain protein concentration (GPC) is one of the most important factors influencing pasta-making quality. Durum wheat varieties with high GPC produce pasta with greater cooked firmness and increased tolerance to overcooking. However, genetic improvement of this important trait has been slowed by the large environmental effect on expression of GPC and the negative correlation with grain yield. Understanding the genetics and identification of molecular markers associated with high GPC could help durum wheat breeders to select for high GPC breeding lines in earlier generations. The objectives of this study were to identify and validate molecular markers associated with quantitative trait loci (QTL) for elevated GPC in durum wheat. A genetic map was constructed with SSR and DArT® markers in an F_1-derived doubled haploid population from the cross DT695 x Strongfield. GPC data were collected from replicated trials at six Canadian environments from 2002 to 2005. QTL associated with variation for GPC were identified on the group 1, 2, and 7 chromosomes and on 5B and 6B, but only *QGpc.usw-B3* on 2B and *QGpc.usw-A3* on 7A were expressed consistently and significantly (LOD>3.0) at four and six environments, respectively. Positive alleles for GPC at these loci were contributed by the higher-GPC parent Strongfield. The *QGpc.usw-A3* QTL was validated in a second DH population, and depending on environment, selection for the Strongfield allele resulted in +0.4% to +1.0% increase in GPC, with little effect on yield in most environments. Given the consistent expression pattern in multiple populations and environments, *QGpc.usw-A3* could be useful for marker-assisted selection for high GPC in durum wheat breeding programs.

[*)] This chapter is already published in Theoretical and Applied Genetics, August 2009, 119: 437-448.

3.2. Introduction

Grain protein concentration (GPC) and gluten quality of durum wheat (*T. turgidum* L. var. *durum*) are the most important factors influencing end-use suitability of durum semolina for pasta products (Distelfeld *et al.*, 2006; Gonzalez-Hernandez *et al*., 2004). High GPC is associated with good pasta cooking firmness and tolerance to over-cooking, particularly at high pasta drying temperatures (Autran *et al*., 1986; D'Egidio *et al*., 1990; Feillet and Dexter, 1996). Semolina protein concentration accounts for 30 to 40% of the variability in pasta cooking quality (Dexter and Matsuo, 1977). Given the importance of GPC, genetic improvement has been the target of durum wheat breeding programs worldwide (Olmos *et al*., 2003), but genetic improvement has been slow, largely because of the inverse correlation between GPC and grain yield (Steiger *et al*., 1996; Cox *et al*., 1985b). Expression of GPC is also strongly influenced by environment (Blanco *et al*., 2006). Adequate nitrogen fertilization and appropriate application timing can be used to elevate GPC (Feillet, 1988), but the continued use of fertilizers is becoming less economical. Hence, development of varieties that are genetically predisposed to higher GPC is a better alternative.

In hexaploid (*T. aestivum* L.) and durum wheat, the inheritance of GPC is complex with quantitative trait loci (QTL) reported on chromosomes 1B and 6A (Perretant *et al*., 2000), on the group 2 and 7 chromosomes (Dholakia *et al*., 2001; Prasad *et al*., 2003; Groos *et al*., 2003) and on 3A (Prasad *et al*., 2003; Groos *et al*., 2003), 4A and 6B (Prasad *et al*., 2003) and 4D (Groos *et al*., 2003). In durum wheat, Blanco *et al*. (1996) reported QTL for GPC on 4BS, 5AL, 6AS, 6BS and 7BS. In addition, the high protein locus *Gpc-B1* on 6B derived from *T. turgidum* L. var. *dicoccoides* has been cloned (Uauy *et al*., 2006a), and increases GPC by up to 1.5% with non-significant effects on protein quality, plant height, heading date, or yield in near isogenic backgrounds (Chee *et al*., 2001). This gene is also associated with increased grain zinc, and iron content and is involved in leaf senescence (Uauy *et al*., 2006a).

In tetraploid wheat, most QTL have been identified from *T. turgidum* L. var. *dicoccoides* (Blanco *et al*., 2002; Olmos *et al*., 2003; Gonzalez-Hernandez *et al*., 2004; Blanco *et al*., 2006) and their use in Canadian breeding programs has been limited,

either because they do not improve protein concentration levels (Kovacs *et al*., 1998) or because of potentially negative effects on other important traits due to excessive linkage drag (Colmer *et al*., 2006). Thus, identification of QTL associated with GPC in well adapted genetic backgrounds would be useful. Strongfield, a Canadian durum wheat variety (Clarke *et al*., 2005), has consistently displayed high levels of GPC coupled with high yield in Canadian environments and has been used extensively in durum wheat crossing programs worldwide.

Marker assisted selection has been suggested to enhance selection accuracy and efficiency especially for trait with complex inheritance, such as GPC. There have been numerous reports on QTL for economic traits of durum wheat, but relatively few markers have actually been validated. The objectives of this study were to identify and validate useful molecular markers associated with elevated GPC in durum wheat that could aid durum wheat breeders to select for this important trait in earlier generations.

3.3. Materials and methods

3.3.1. Plant material and trait evaluation

One hundred and eighty five F_1-derived doubled haploid (DH) lines from the cross DT695 x Strongfield (Figure 3.1; Clarke *et al*., 2005) were grown along with their parents in two replicate field trials in an alpha-lattice design at Regina (RG) and Swift Current (SC) in 2002; Saskatoon (ST), Regina and Swift Current in 2003; and Saskatoon in 2005. Twenty four blocks each containing eight genotypes were nested in each replicate. All locations are in Saskatchewan, Canada. DT695 is derived from the cross DT471/2*Kyle (Figure 3.1). Kyle is a Canadian durum wheat cultivar developed by Agriculture and Agri-Food Canada (Townley-Smith *et al*. 1987). The doubled haploid lines were generated using the maize pollen procedure described by Knox *et al*. (2000). At maturity (ZGS 9.0; Zadocks *et al*., 1974), plots were harvested with a small-plot combine and dried to approximately 9% moisture. Yield was converted to a kg ha^{-1} based on area harvested. Grain protein concentration (%) was determined on whole grain samples from individual plots using a FOSS-6500 Near Infrared Reflectance Spectrophotometer (NIRS) calibrated with reference samples analyzed for GPC using a Leco-N Analyzer (LECO FP-528). Prediction of GPC by NIRS was confirmed by

analysis of 30 samples selected at random from the field trials of 2003 and 2005 using Leco-N analysis. The seed weight of 1000 kernels (g) was also determined for each plot.

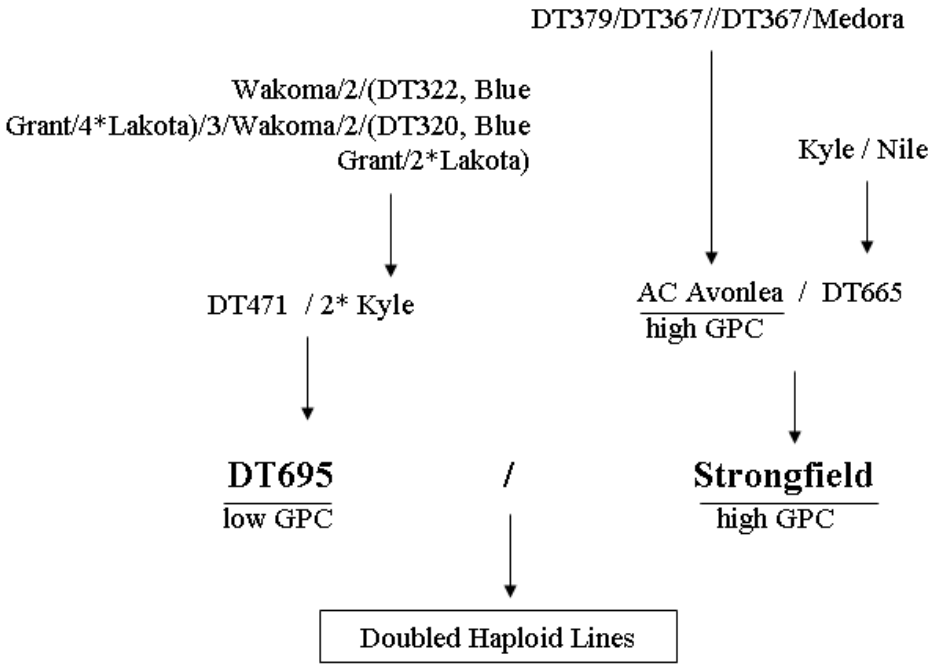

Figure 3.1. The pedigree of the DH mapping population used for GPC QTL identification.

3.3.2. Statistical analysis of phenotypic data

For each environment, analysis of variance (ANOVA) was performed using PROC MIXED of SAS (SAS Institute Inc., 2003) where genotypes were considered fixed effects, and replications and blocks as random effects. Genetic variance was estimated by performing the same analysis but with genotypes considered random. For each environment, phenotypic variance (σ^2_p) was estimated as the sum of genetic variance (σ^2_g) and average variance estimate of residual (σ^2_e), such that $\sigma^2_p = (\sigma^2_g + \sigma^2_e/r)$. Heritability was estimated as the proportion of genetic variance to phenotypic variance, such that $h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_e/r)$. Parental data was removed for heritability estimation. Confidence intervals for heritability estimates (h^2) were calculated

according to Knapp *et al*. (1985). The least square (LS) means from each environment were correlated using PROC CORR of SAS (SAS Institute Inc., 2003).

3.3.3. SSR and DArT® marker analysis

For marker analysis, 94 lines from the DH population were randomly selected and the genomic DNA was extracted from two-week old plants using the cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). Simple sequence repeat (SSR) markers showing polymorphism after an initial PCR screening of the parents were screened on the DH population. The SSR included *gwm* (Röder *et al*., 1998), *cfa* and *cfd* (Sourdille *et al*., 2003), *gdm* (Pestsova *et al*., 2000), *barc* (Song *et al*., 2005; the GrainGenes database [http://wheat.pw.usda.gov]), *wmc* Gupta *et al*., 2002; http://wheat.pw.usda.gov/ggpages/SSR/WMC). Reactions were performed in a 96-well PCR plate contained each 25 µl of a reaction mixture of 2.5 µL 10x PCR buffer, 1.5 mM of $MgCl_2$, 0.1 mM of each dNTP, 0.04 µM of M13 sequence-modified forward SSR primer, 0.16 µM of reverse SSR primer, 0.16 µM of Universal dye-labeled M13 primer, 1 U of *Taq* DNA polymerase and 50 ng of genomic DNA. The PCR cycle included an initial denaturation of 3 minutes at 94^0C; followed by 30 cycles of: 30 seconds at 94^0C, 45 seconds annealing, and 45 seconds at 72^0C; and 7 cycles of: 30 seconds at 94^0C, 45 second at 53^0C, 45 seconds at 72^0C; and final extension of 10 minutes at 72^0C. Primer sequences and the annealing temperature were as reported previously (Röder *et al*., 1998, http://wheat.pw.usda.gov). The forward primer of each SSR marker pair was modified by incorporating the M13 sequence to the 5' end during synthesis (Schuelke, 2000). The Universal M13 primer was labeled with either FAM, VIC, NED or PET fluorescent dyes. Amplification products (0.5 µl) were combined with 9.5 µl HiDi formamide (ABI, Foster City, California) and 0.05 µl ROX size standard, and run on a 36 cm capillary electrophoresis (ABI 3130 Genetic Analyzer). The electropherograms were analyzed with GeneMapper version 4.0. Markers with parental allele sizes differing by 8 bp were scored on 2% (w/v) agarose gel stained with 0.5 µg/ml ethidium bromide.

Diversity Array Technology (DArT®) markers generated by Triticarte Pty Ltd (Canberra, Australia; http://www.triticarte.com.au) are a cost-effective means to

generate genetic maps in a number of species (Wittenberg *et al*., 2005) including durum wheat (Pozniak *et al*., 2007) were applied in the DT695 x Strongfield population. The DArT® analysis was performed by Triticarte Pty Ltd, and DH lines were scored for the presence or absence of hybridization based on fluorescence signal intensities. Wheat DArT® markers previously mapped have been named, eg. wPt-8770, but in some cases clone names were used for identification (eg. 304069).

3.3.4. Genetic map and QTL analysis

A genetic linkage map of the DT695 x Strongfield population was constructed using the Haldane mapping function within the software JoinMap® 3.0 (van Ooijen and Voorrips, 2004). To improve map robustness, markers displaying unusually high frequencies of double crossover events and/or segregation distortion were removed prior to final map construction. Final map construction consisted of SSR and DArT® markers joined at a LOD score of 3.0 using the "Second Order" mapping function in JoinMap® 3.0. Linkage groups were assigned chromosome names by comparing markers on the generated map to previously published durum maps (Korzun *et al*., 1999; Nachit *et al*., 2001; Elouafi and Nachit 2004) and the hexaploid wheat SSR consensus map (Somers *et al*., 2004).

Least square (LS) means of GPC from the six environments were used in QTL analysis. Simple interval mapping (SIM) was used first to identify markers most significantly associated with variation in GPC. To enhance the power of QTL detection, the analyses were repeated using those markers identified by SIM as co-factors in a multiple QTL model (MQM) in MapQTL Version 5.0 (van Ooijen and Voorrips, 2004). For each environment, the genome-wide significance threshold ($P<0.01$) of the LOD score was determined as described previously (van Ooijen *et al*., 1999). For each QTL, the average QTL effect (one half the differences between parental marker class means) was estimated by MapQTL. Single factor ANOVA was used to assess marker association with phenotypic variance for those markers not assigned to linkage groups. The MQM-identified QTL were designated as *QGpc.usw* (University of Saskatchewan-Pozniak laboratory designation) as per the recommended rules for gene symbolization in wheat.

3.3.5. Marker validation

A second population consisted of one hundred and ten F1-derived doubled haploid (DH) lines from the cross 9370-DJ**3 x Strongfield, along with their parents, were used to validate QTL identified in the DT695 x Strongfield population. The parent 9370-DJ**3 is a breeding line developed at the Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Swift Current, Saskatchewan. The GPC data were obtained using NIRS on samples collected from each plot of two replicate-field trials grown in an alpha-lattice design at Regina and Swift Current in 2002, and at Regina, Swift Current and Saskatoon in 2003. Yield data was assessed on a plot basis and converted to kg ha^{-1} based on the plot area harvested. Data for each environment were analyzed separately to generate LS means using PROC MIXED of SAS (SAS Institute Inc., 2003) where genotypes were considered as fixed effects, and replications and blocks as random effects. The SSR markers linked to stable QTL identified in the DT695 x Strongfield population were analyzed against GPC LS means from the five environments using a single factor ANOVA with each marker considered as a fixed effect.

3.4. Results

3.4.1. Environmental conditions

For this study, the two populations used for genetic analysis of GPC were evaluated in environments with very different environmental conditions (Table 3.1). In 2002 all test sites received above average precipitation, particularly in June when plants were tillering, and during grain fill in August. In 2003, below average precipitation coupled with above average temperatures in July and August resulted in extreme drought stress at all three environments. Above average precipitation at Saskatoon in June 2005 coupled with below average temperatures throughout the growing season delayed plant development and maturity compared to other environments.

Table 3.1. Growing season precipitation (mm month^{-1}) and average monthly temperatures (°C) in six environments used to evaluate the Strongfield x DT695 and validation mapping populations. Average precipitation and monthly temperature at each environment were adapted from http://www.climate.weatheroffice.ec.gc.ca/climateData/dailydata_e.html

	Average precipitation (mm month^{-1})						**Average monthly temperatures (°C)**				
Environment[†]	**May**	**June**	**July**	**Aug**	**Sept**	**Total**	**May**	**June**	**July**	**Aug**	**Sept**
SC 2002	12	123	73	102	59	369	8.7	15.7	19.5	15.3	12.0
RG 2002	9	129	29	113	38	318	8.0	16.3	20.0	16.2	11.9
RG 2003	31	31	42	12	25	141	11.6	16.0	19.8	20.9	11.5
SC 2003	41	78	8	20	31	178	10.7	15.1	19.7	21.3	11.8
ST 2003	14	31	64	31	25	165	11.8	15.9	18.2	20.6	11.3
ST 2005	31	193	53	54	74	405	10.2	14.4	17.5	15.4	11.3
30 Years Average *)											
SC	44	66	52	40	28	230	11.1	15.6	18.1	17.9	11.8
RG	52	65	68	38	33	256	11.6	16.3	18.5	17.4	10.9
ST	42	61	57	35	29	224	11.8	16	18.3	17.6	11.5

[†]RG = Regina, SC = Swift Current, ST = Saskatoon

*) adapted from http://www.worldweather.org/056/c00628.htm

3.4.2. Phenotypic data

Grain protein concentration (%) data for the DT695 x Strongfield mapping population were collected using near-infrared spectroscopy (NIRS) following calibration with reference samples. To validate the use of NIRS, 30 random plot samples were chosen from each environment and measured with Leco-N Analyzer (LECO FP-528). The NIRS GPC data showed a high correlation (range 0.95-0.97; P<0.01) with the LECO generated GPC data (Table 3.2).

Table 3.2. Pearson correlation coefficients between the NIRS and LECO GPC data collected in 2003 and 2005 testing environments.

RG 2003 †	ST 2003	SC 2003	ST 2005
0.97**	0.95**	0.97**	0.97**

†RG = Regina, SC = Swift Current, ST = Saskatoon
** = significant at the level of 0.01 of probability

In the combined ANOVA over all environments, the GxE interaction was significant (P < 0.01) (Appendix 3.1). As such, data is presented for each environment. The ANOVA for GPC revealed significant differences (P<0.01) among DH lines at all environments. Across environments, GPC ranged from 11.1 to 16.6% for DT695 and from 13.3 to 17.6% for Strongfield (Table 3.3). Strongfield had significantly higher (P<0.05) GPC than DT695 in all environments except at RG 2002 where GPC of Strongfield was only numerically higher (Table 3.3).

At all environments, bi-directional transgressive segregation was evident for GPC (Figure 3.2) with the lowest transgressive segregant being significantly lower than the low-GPC parent in three environments. The range in GPC was lowest at SC 2003 (3.1%), and highest at RG 2002 (5.0%) with an average range of 3.7% (Table 3.3). At all environments, the GPC LS means were normally distributed based on the Shapiro-Wilk test (Figure 3.2, Appendix 3.3). The genetic variance of GPC across environments ranged from 0.20 to 0.53%, with heritability estimates ranging from 0.51 to 0.70 (Table 3.3). Correlation coefficients of LS means among environments ranged from 0.23 to 0.68 (Table 3.4), consistent with strong environmental influence on phenotypic expression of GPC.

Table 3.3. Least square means of GPC (%) for Strongfield and DT695, and high and low transgressive segregants observed across environments in the DT695 x Strongfield mapping population. The genetic variances (σ^2_g) (%) and heritability estimates (h^2) and 95% confident intervals for each environment are also presented.

	RG 2002†	SC 2002	ST 2003	RG 2003	SC 2003	ST 2005
DT695	13.9	11.1	16.6	13.3	15.9	13.2
Strongfield	14.9	13.3	17.6	14.8	17.1	14.4
Low transgressive	12.8	11.0	15.5	12.7	15.4	12.0
High transgressive	17.8	14.4	18.8	16.3	18.5	15.4
Population mean	14.7	12.8	17.0	14.2	16.5	14.2
σ^2_g	0.50	0.49	0.25	0.53	0.20	0.26
h^2	0.68	0.67	0.65	0.51	0.61	0.70
h^2 95% CI	0.55-0.77	0.56-0.75	0.53-0.74	0.35-0.63	0.48-0.71	0.59-0.77
LSD ($P < 0.05$)	1.1	1.0	0.8	1.0	0.9	0.9

†RG = Regina, SC = Swift Current, ST = Saskatoon

Table 3.4. Pearson correlation coefficients of GPC LS means of the DT695 x Strongfield mapping population observed across environments.

	RG 2002†	SC 2002	ST 2003	RG 2003	SC 2003
SC 2002	0.58 **				
ST 2003	0.43 **	0.52 **			
RG 2003	0.38 **	0.56 **	0.68 **		
SC 2003	ns	0.23 **	0.44 **	0.28 **	
ST 2005	0.39 **	0.37 **	0.54 **	0.50 **	0.24 **

†RG = Regina, SC = Swift Current, ST = Saskatoon
** = significant at the level of 0.01 of probability
ns = not significant

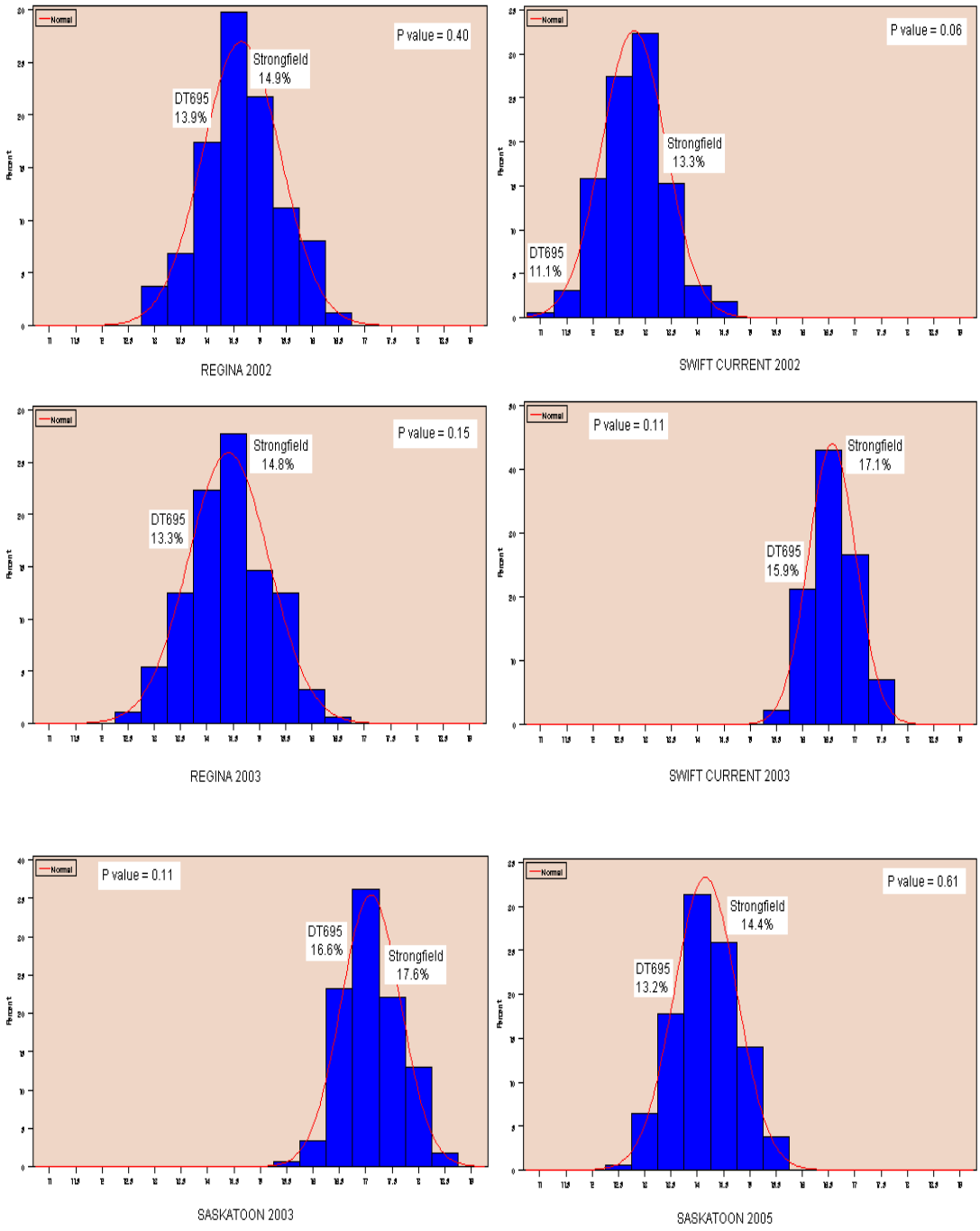

Figure 3.2. Distribution and normality of Least Square means of GPC among the DH lines of the DT695 x Strongfield mapping population over six environments. P value greater than 0.05 means that the data are normally distributed.

The ANOVA showed that grain yields of Strongfield and DT695 were variable across environments, with Strongfield out-yielding DT695 only at SC 2003 and ST 2005 (Table 3.5). In contrast, DT695 produced statistically ($P<0.05$) more grain than Strongfield at RG 2003 (Table 3.5). Average grain yields were less in 2003, likely because of drought conditions (Table 3.1). The ANOVA indicated significant differences in grain yield among the DH lines in all environments (Appendix 3.2) and large transgressive segregation was evident. The greatest range in yield was at ST 2005 (2149 kg ha^{-1}) and RG 2002 (2523 kg ha^{-1}), but the latter site had higher residual variation compared to other sites (Table 3.5). Likewise, 1000-kernel weights were highly variable and ranged from 31.0 to 51.4 g per thousand kernels for DT695 and from 30.1 to 47.9 g per thousand kernels for Strongfield (Table 3.5). Strongfield grains weighted less than DT695, but differences were only significant at SC 2002, RG 2003, and ST 2005. Large transgressive segregation for 1000-kernel weight was evident among the DH lines of the mapping population (Table 3.5). The range in 1000-kernel weight was lowest at ST 2005 (11.0 g per thousand kernels) and highest at ST 2003 (16.3 g per thousand kernels). Despite the large range in 1000-kernel weight, significant negative correlations were observed between GPC and 1000-kernel weight only at SC 2002 and ST 2005 (Table 3.5). In contrast, grain yield was negatively correlated with GPC at all six environments, with r values ranging from -0.45 to -0.51 ($P<0.01$; Table 3.5).

3.4.3. Genetic map and QTL analysis

A total of 488 SSR markers were scored on the parents of the mapping population and 190 (40%) markers produced polymorphic fragments. Approx. 260 DArT® markers were polymorphic and scored in the DH population. The final genetic map was constructed based on "Second-order mapping function" which is a conservative test of linkage. It consisted of 140 SSR and 205 DArT® markers that were joined into 25 linkage groups. Twenty-four linkage groups could be assigned to a chromosome based on previously published genetic maps with the remaining linkage group consisting of 13 tightly linked DArT® markers and spanning approx. 30 cM.

Table 3.5. Least square means of grain yield (kg ha^{-1}) and 1000-kernel weight (g) for the DT695 x Strongfield population, Strongfield and DT695, and high and low transgressive segregants across six environments. The Pearson correlation coefficients between grain protein concentration, yield and 1000-kernel weight for this population across environments are also presented.

Factor	1000-kernel weight (g)						Grain Yield (kg ha^{-1})					
	RG 2002[†]	SC 2002	ST 2003	RG 2003	SC 2003	ST 2005	RG 2002	SC 2002	ST 2003	RG 2003	SC 2003	ST 2005
DT695	37.7	44.0	47.3	51.4	31.0	46.8	4651	3285	2397	3321	1759	6055
Strongfield	35.0	39.7	46.7	47.9	30.1	42.2	5013	3329	2246	2932	2008	6757
High transgressive	42.2	48.3	54.4	56.3	37.0	50.7	5362	4047	2819	3723	2365	6928
Low transgressive	30.7	36.0	38.1	41.3	25.5	39.7	2839	2225	1567	2197	1348	4779
Population mean	36.2	41.7	46.7	48.9	31.5	43.6	4313	3265	2355	2889	1856	6007
LSD (P<0.05)	4.2	3.1	3.6	2.6	2.2	3.7	928	487	470	380	339	510
GPC Correlation (r)	-0.16*	ns	ns	ns	ns	-0.33*	-0.57**	-0.51**	-0.46**	-0.59**	-0.45**	-0.48**

[†]RG = Regina, SC = Swift Current, ST = Saskatoon

* = significant at the level of 0.05 probability; ** significant at the level 0.01 probability; ns = not significant

This group could not be assigned to a chromosome. Chromosomes 1B and 3B were the only two chromosomes represented by a single linkage group, with the remaining represented by two linkage groups, one for each chromosome arm. The order of SSR markers was in agreement with previously published wheat genetic maps (Groos *et al*., 2003; Elouafi and Nachit, 2004; Somers *et al*., 2004; Blanco *et al*., 2006). The final linkage map spanned 1474 cM, similar to previously published durum maps (Pozniak *et al*., 2007).

Using MQM, nine QTL for GPC were identified (Figure 3.3). The average effects of individual QTL ranged from 0.16-0.46% (Table 3.6), and no significant two-way interactions between QTL were identified. A QTL x environment interaction was evident for GPC in this population with six of the QTL being significant at only one environment (*QGpc.usw-A1* on 1A, *QGpc.usw-B1* on 1B, *QGpc.usw-B2* on 1B, *QGpc.usw-B4* on 5B *QGpc.usw-B5* on 6B, *QGpc.usw-B6* on 7B) (Table 3.6). The *QGpc.usw-A2* on 2A was significant at three environments (Table 3.6). DT695 contributed the allele for elevated protein at four of these QTL (*QGpc.usw-B2*, *QGpc.usw-A2*, *QGpc.usw-B5* and *QGpc.usw-B6*; Figure 3.3), whereas Strongfield contributed alleles for elevated GPC at five of the QTL (*QGpc.usw-A1, QGpc.usw-B1, QGpc.usw-B3, QGpc.usw-B4, and QGpc.usw-A3*; Figure 3.3). The QTL *QGpc.usw-B3* on 2B and *QGpc.usw-A3* on 7A, were significant in five and six out of the six environments evaluated, respectively (Table 3.6). The *QGpc.usw-B3* was flanked by *wPt-0694* and *wmc41* and spanned approximately 11 cM and its effect ranged from 0.20% at SC 2002 and SC 2003 to 0.26% at RG 2002. The *QGpc.usw-A3* QTL centered at *barc281* and spanned approx. 10 cM (Figure 3.3) with a QTL effect ranging from 0.18% at ST 2005 to 0.46% at RG 2003 and was flanked by *gwm4* and *barc108.* Strongfield, contributed alleles for elevated GPC at both *QGpc.usw-B3* and *QGpc.usw-A3* (Figure 3.3). None of the markers excluded from the genetic linkage map were significantly associated with grain protein.

To determine if the GPC QTL identified were associated with variation in grain yield and 1000-kernel weight, single marker analysis was performed for those markers identified as being most associated with variation in GPC. Single marker analysis for 1000-kernel weight revealed that only markers at the *QGpc.usw-B2*, *QGpc.usw-B4* and

Table 3.6. QTL associated with variation in GPC in the DT695 x Strongfield doubled haploid population. QTL effects are presented for markers closest to the centre of the QTL.

Chromosomes	QTL	RG 2002		SC 2002		RG 2003		SC 2003		SK 2003		SK 2005	
		LOD	Effect	LOD	Effect	LOD	Effect	LOD	Effect	LOD	Effect	LOD	Effect
1A	*QGpc.usw-A1*	ns[b]	-	ns	-	ns	-	3.2**	0.19	ns	-	ns	-
1B	*QGpc.usw-B1*	ns	-	ns	-	3.8**	0.24	ns	-	ns	-	ns	-
1B	*QGpc.usw-B2*	ns	-	ns	-	ns	-	5.6**	0.27	ns	-	ns	-
2A	*QGpc.usw-A2*	4.9**	0.35	4.1**	0.23	ns	-	5.1**	0.22	ns	-	ns	-
2B	*QGpc.usw-B3*	2.6**	0.26	3.4**	0.20	ns	-	3.7**	0.20	ns	-	4.8**	0.22
5B	*QGpc.usw-B4*	ns	-	ns	-	ns	-	ns	-	3.9**	0.19	ns	-
6B	*QGpc.usw-B5*	ns	-	ns	-	ns	-	ns	-	3.6**	0.19	ns	-
7A	*QGpc.usw-A3*	4.9**	0.36	9.5**	0.37	10.5**	0.46	2.4*	0.16	8.5**	0.32	3.0**	0.18
7B	*QGpc.usw-B6*	ns	-	ns	-	ns	-	ns	-	ns	-	3.1**	0.20

†RG = Regina, SC = Swift Current, ST = Saskatoon

[a] Parent contributing the positive allele (high GPC) at each QTL where St = Strongfield and DT = DT695.

[b] ns not significant at the 5% significance level, **LOD score significant at the 1% level, *LOD significant at the 5% level

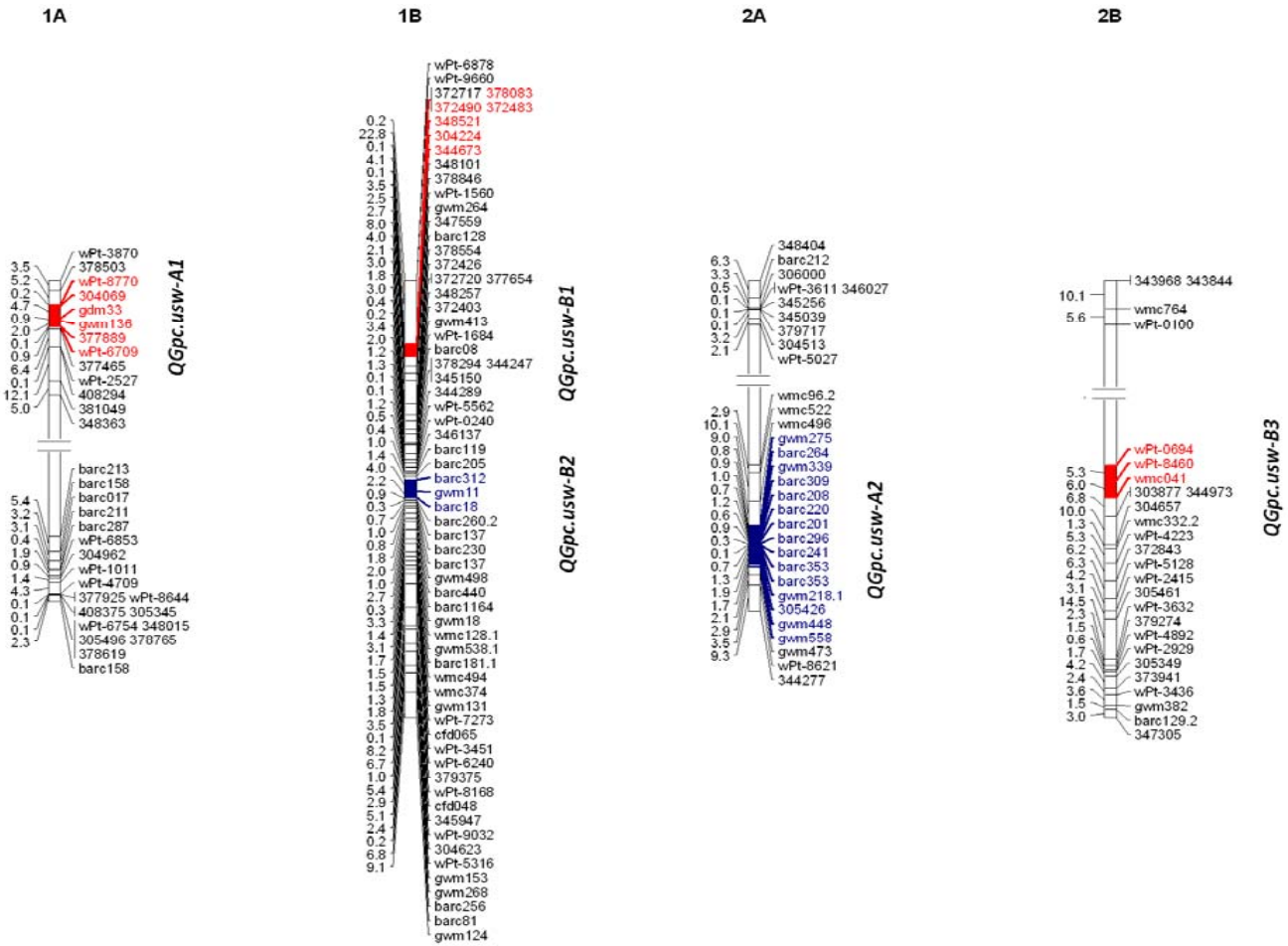

Figure 3.3. QTL associated with GPC identified in the DT695 x Strongfield population. Red boxes indicate that the allele effect is contributed by Strongfield, while blue boxes indicate that the allele effect is contributed by DT695. Values in the left hand side of the chromosomal bars indicate the genetic distance (cM) between markers. Notations in the right hand side of chromosomal bars indicate the molecular markers

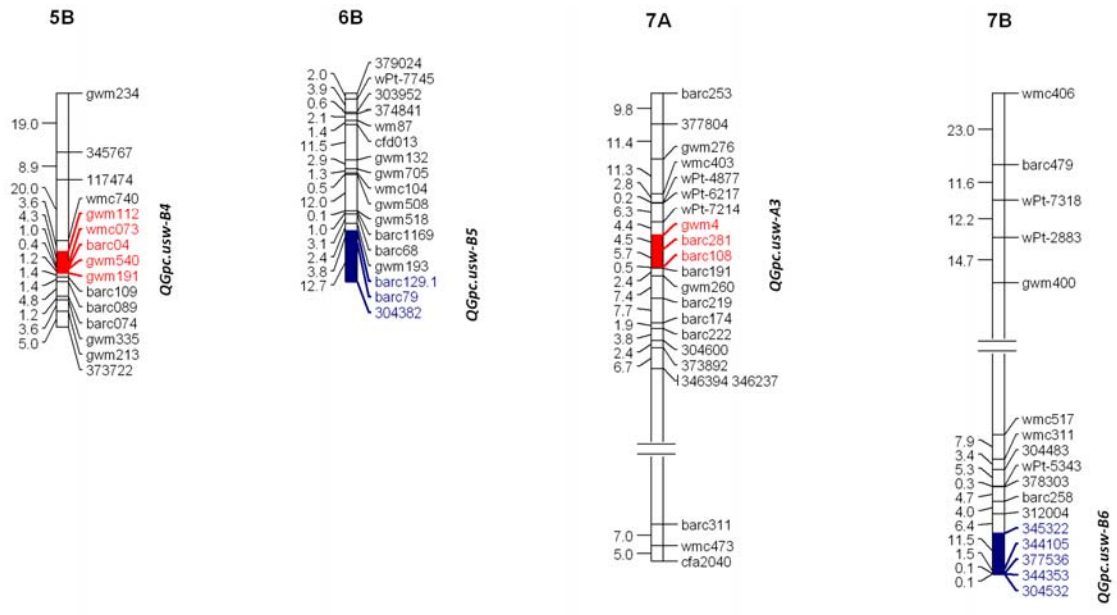

Figure 3.3. (continued from page 42)

QGpc.usw-B5 were associated with 1000-kernel weight variation (Table 3.7). The *QGpc.usw-B2* was also associated with variation in grain yield at ST in both 2003 and 2005. The *QGpc.usw-B4* QTL was associated with 1000-kernel weight at five ($P<0.05$) of the six environments evaluated and the allele for reduced 1000-kernel weight was contributed by Strongfield, the high protein parent. The two major GPC QTL *QGpc.usw-B3* and *QGpc.usw-A3* were not associated with 1000-kernel weight in any of the environment evaluated (Table 3.7). However, Strongfield alleles at *QGpc.usw-B3* and *QGpc.usw-A3* were associated with reduced 1000-kernel weight, but each only in a single environment (Table 3.7)

3.4.4. Marker validation

In the 9370-DJ**3 x Strongfield validation population, ANOVA for GPC using a mixed linear model revealed highly significant differences ($P<0.01$) among DH lines across environments (Appendix 3.4). When combined over environments, the GxE interaction was significant (Appendix 3.5). As such, data is presented for each environment. Across environments, GPC ranged from 11.9 to 17.8% for 9370-DJ**3 and from 13.3 to 17.6% for Strongfield (Table 3.8). Significant differences in GPC between the parents could only be observed at RG 2002, but bi-directional transgressive segregation in this population was evident across environments (Table 3.8; Figure 3.4). The range in GPC was lowest at RG 2002 (3.4%), and highest at SC 2002 (5.4%) (Table 3.8) with an average range of 4.3%. Correlation coefficients of LS means among sites ranged from 0.27 to 0.67 (Table 3.9). At all environments, the GPC LS means were normally distributed based on the Shapiro-Wilk test (Figure 3.4; Appendix 3.6). Grain yield was statistically similar ($P>0.05$) between parents, except at SC 2002 where Strongfield produced more grain (Table 3.8). However, yield was variable in the DH population with statistical differences ($P<0.01$) between high and low transgressive segregants at all sites (Table 3.8). As in the DT695 x Strongfield population, there was a strong negative correlation between grain protein and grain yield with r values ranging from -0.33 to -0.75 (Table 3.10).

Given the consistent expression of *QGpc.usw-B3* and *QGpc.usw-A3* in the DT695 x Strongfield population (See section 3.4.3), markers linked to these QTL were

Table 3.7. Single factor ANOVA for association between yield (kg ha^{-1}) and 1000-kernel weight (g) and markers significantly associated with grain protein concentration QTL in the DT695 x Strongfield doubled haploid mapping population (See Table 3.6). Only markers with significant F-tests are presented.

			F-values					
GPC QTL : markers	Chrom.	Trait	SC 2002[†]	RG 2002	ST 2003	SC 2003	RG 2003	ST 2005
QGpc.usw-A1:	1A	Yield	ns	ns	ns	ns	ns	ns
gdm33		*KWT*	ns	ns	ns	ns	ns	ns
QGpc.usw-B2:	1B	*Yield*	ns	ns	23.28**	ns	ns	18.06**
barc18		*KWT*	9.13 **	ns	9.05 **	18.63**	ns	ns
QGpc.usw-A2:	2A	*Yield*	6.11*	ns	ns	ns	7.50**	ns
barc201		*KWT*	ns	ns	ns	ns	ns	ns
QGpc.usw-B3:	2B	*Yield*	ns	ns	ns	ns	ns	5.62*
wmc41		*KWT*	ns	ns	ns	ns	ns	ns
QGpc.usw-B4:	5B	*Yield*	ns	ns	ns	ns	ns	ns
wmc73		*KWT*	6.89 *	5.49 *	ns	10.13 **	5.08 *	8.52 **
QGpc.usw-B5:	6B	*Yield*	ns	ns	ns	ns	ns	ns
barc79		*KWT*	8.14 **	ns	6.08 *	ns	5.07 *	ns
QGpc.usw-A3:	7A	*Yield*	ns	ns	ns	ns	15.4**	ns
barc108		*KWT*	ns	ns	ns	ns	ns	ns

[†]RG = Regina, SC = Swift Current, ST = Saskatoon
* = significant at the level of 0.05 probability
** = significant at the level of 0.01 probability
ns = not significant

Table 3.8. Least square means of grain protein concentration (%) and grain yield (kg ha-1) in the 9370-DJ**3 x Strongfield validation population, its parents, and high and low transgressive segregants.

	Grain protein concentration (%)					Yield (kg ha^{-1})				
	RG 2002†	SC 2002	RG 2003	SC 2003	ST 2003	RG 2002†	SC 2002	RG 2003	SC 2003	ST 2003
Strongfield	14.5	13.1	16.3	17.0	17.8	4573	3558	2794	1986	2620
9370-DJ**3	12.8	12.3	16.5	18.0	17.8	5134	2894	2981	1857	2510
High transgressive	15.8	16.4	19.1	19.8	19.9	5711	4227	3748	2504	3201
Low transgressive	12.4	11.0	14.2	16.0	15.9	2847	1783	1739	1323	1784
Population mean	13.9	12.8	16.6	17.7	17.8	4538	3094	2797	1906	2512
LSD (P<0.05)	0.8	1.0	0.9	1.2	0.6	647	660	301	238	379

†RG = Regina, SC = Swift Current, ST = Saskatoon

Table 3.9. Pearson correlation coefficients of GPC LS means of the 9370-DJ**3 x Strongfield validation population observed across environments.

	RG 2002†	SC 2002	RG 2003	SC 2003
SC 2002	0.52 **			
RG 2003	0.53 **	0.34 **		
SC 2003	0.44 **	0.27 **	0.60 **	
ST 2003	0.47 **	0.32 **	0.67 **	0.61 **

†RG = Regina, SC = Swift Current, ST = Saskatoon
** = significant at the level of 0.01 of probability

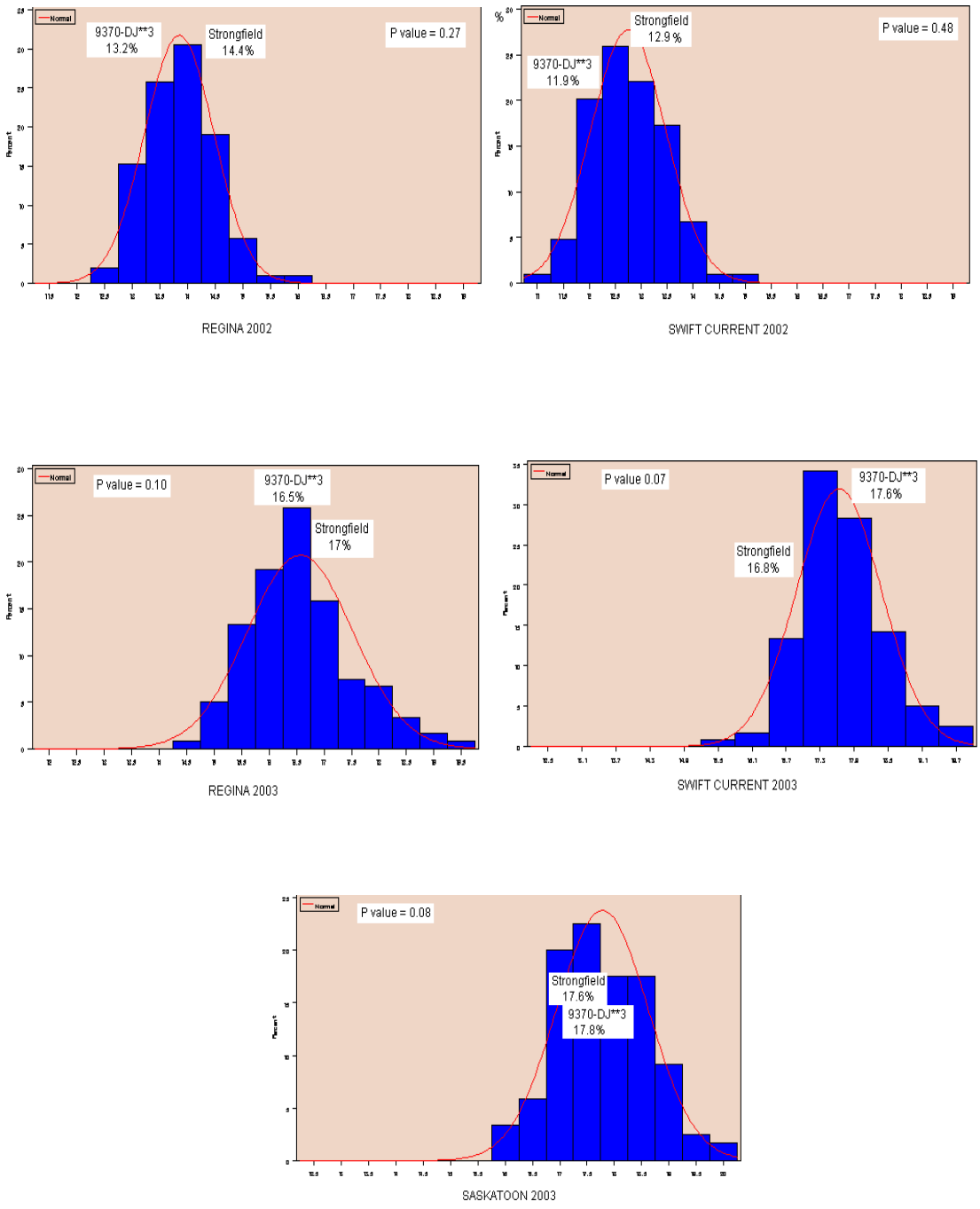

Figure 3.4. Distribution and normality of Least Square means of GPC among the 9370-DJ**3 x Strongfield validation population over five environments. P value greater than 0.05 means that the data are normally distributed.

validated against GPC LS means of the 9370-DJ**3 x Strongfield population from five locations for marker-QTL association validation. Of the markers linked to *QGpc.usw-A3* (Figure 3.3), only *barc108* was polymorphic but consistently showed significant (P<0.01) association with high GPC in the validation population (Table 3.10). The effects of the Strongfield allele at *barc108* ranged from +0.4% at RG 2002 to +1.0% at ST 2003 (Table 3.10). Despite the association with GPC in this population at all environments, *barc108* was associated with variation in grain yield only at RG 2003 and ST 2003 (Table 3.10). At those sites, DH lines carrying the Strongfield allele had 226 kg ha^{-1} (RG 2003) and 213 kg ha^{-1} (ST 2003) lower yield than lines carrying the 9370-DJ**3 allele (Table 3.10). Of the DH lines carrying the Strongfield allele, 58% had yield and GPC similar or greater than Strongfield at RG 2003. Similarly, 72% had similar or greater yield and GPC than Strongfield at ST 2003 (Table 3.10). None of the markers at *QGpc.usw-B3* (Figure 3.3) were polymorphic in the validation population, and thus the QTL could not be verified.

Table 3.10. Single factor ANOVA for *barc108* and GPC and yield in the 9370-DJ**3 x Strongfield validation population. Least square means for genotypes homozygous for *barc108* (*QGpc.usw-A3*) and standard error of the difference (SED) between the two marker classes are presented.

	RG 2002	SC 2002	RG 2003	SC 2003	ST 2003
barc108 F-test-GPC	5.65**	9.16**	12.76**	5.51*	22.56**
barc108 F-test-yield	ns	ns	10.58**	ns	13.63**
Yield-GPC Correlation (r)	-0.33**	-0.75**	-0.75**	-0.71**	-0.68**
	barc108 class Least square means				
Strongfield allele					
GPC (%)	14.0	13.0	17.0	17.8	18.2
Yield (kg ha^{-1})	4512	3110	2680	1870	2400
Proportion	81%	79%	58%	81%	72%
9370-DJ3 allele**					
GPC (%)	13.6	12.4	16.1	17.3	17.2
Yield (kg ha^{-1})	4614	3220	2906	1927	2613
GPC SED (%)	0.14	0.19	0.23	0.18	0.20
Yield SED (kg ha^{-1})	149	76	118	41	68

†RG = Regina, SC = Swift Current, ST = Saskatoon, * = significant at the level of 0.05 probability, ** = significant at the level of 0.01 probability

3.5. Discussion

The present study was initiated to identify QTL associated with elevated GPC in an adapted durum wheat background, as most QTL reported to date in durum wheat are derived from *T. turgidum* L. var. *dicoccoides***.** Blanco *et al*. (2002) reported seven QTL for elevated GPC from *T. dicoccoides* on 4BS, 5AL, 6AS (two loci), 6BS, 7AS, and 7BS. Unfortunately, six out of seven QTL were associated with reduced grain yield with the exception of the 6B QTL (Blanco *et al*., 2002). The *Gpc-B1* locus on 6BS from *T. dicoccoides* (Olmos *et al*., 2003; Distelfeld *et al*., 2004; Distelfeld *et al*., 2006; Uauy *et al*., 2006) is the most studied and has been suggested as an effective gene to be used for elevation of GPC. The effect of this gene is independent of protein quality, plant height, heading date, and yield (Chee *et al*., 1998) and durum recombinant lines carrying this gene have shown improved GPC (Kovacs *et al*., 1998). However, this locus did not provide protein genes superior to those already available in Canadian durum wheat germplasm (DePauw *et al*., 1998; Kovacs *et al*., 1998). These results suggest that other more effective genes for GPC exist, and efforts to identify additional genes for high GPC in adapted backgrounds are warranted.

Validation of the use of NIRS for assessing GPC was confirmed with the high correlation of GPC of the 30 random plot samples chosen from each environment and measured with both the NIRS and LECO-N Analyzer. The high correlation between NIRS and LECO generated GPC consistent with earlier studies (Long *et al*., 2008) and confirmed NIRS data were reliable for predicting GPC for the mapping study.

The effect of GxE interactions was significant for GPC (Appendix 3.1) as shown by the low correlation of LS means among environments (Table 3.4), and also by the variable expression of QTL in different environments (Table 3.6). This was not surprising given the dramatically different environmental conditions observed over the three years of testing (Table 3.1), and as shown by the moderate heritability of GPC (Table 3.3).

Numerous reports have shown that GPC negatively correlates with 1000-kernel weight and grain yield likely because of protein dilution by starch content (Groos *et al*., 2003, Cox *et al*., 1985b). The highest GPC is usually found under adverse conditions during grain fill since starch synthesis and its re-deposition to the grain is more sensitive

to adverse growing conditions than that of protein (Campbell *et al.,* 1981). Significant negative correlations between GPC and 1000-kernel weight at SC 2002 and ST 2005 were evident (Table 3.5) indicative of the presence of genetic factors segregating in the population that are likely pleiotropic on GPC. Despite the negative correlations between GPC and 1000-kernel weight, only *QGpc.usw-B2*, *QGpc.usw-B4* and *QGpc.usw-B5* were associated with 1000-kernel weight (Table 3.7). Of these three, *QGpc.usw-B4* was associated with 1000-kernel weight in nearly all environments tested (Table 3.7) and the allele for reduced 1000-kernel weight was contributed by Strongfield, contributor of the high GPC allele at this locus (Figure 3.3). Thus *QGpc.usw-B4* is likely not associated with GPC *per se*, but is likely associated with grain protein dilution by the reduced starch content in smaller seeds from Strongfield. The allele for reduced 1000-kernel weight at *QGpc.usw-B2* and *QGpc.usw-B5* were contributed by DT695 (Figure 3.3). In most environments, DT695 had larger seed weight than Strongfield (Table 3.5), and thus these QTL are likely associated with 1000-kernel weight and have a corresponding pleiotropic effect on GPC.

In this study, Strongfield showed an average 1.4% ($P<0.05$) higher GPC compared to DT695 (Table 3.3), consistent with an earlier report of Strongfield expressing high GPC (Clarke *et al*., 2005). Bidirectional transgressive segregation for GPC was observed in the DT695 x Strongfield mapping population (Table 3.3), and indicates that neither Strongfield nor DT695 carry all of the desirable alleles for elevated GPC. This was confirmed by the QTL analysis showing QTL contributions to GPC from both parents (Figure 3.3). However, at RG 2003 only QTL with positive effects from Strongfield were identified (Table 3.6, Figure 3.3), despite a greater than 3% range in GPC at that environment (Table 3.3). This implies that additional smaller effect QTL maybe segregating in this population that were not identified either because of the variability in phenotypic data or due to lack of marker coverage in some genomic regions (Figure 3.3). Only 1B and 3B had adequate marker coverage to form a single linkage group, with the remaining chromosomes represented by at least two linkage groups. Addition of molecular markers in the regions not adequately covered would resolve this hypothesis.

The genetic region for GPC on chromosome 6BS of the wild tetraploid accession Acc. FA-15-3 (*T. turgidum* var *dicoccoides)* (Avivi 1978; Joppa *et al*., 1997; Olmos *et al*., 2003), designated *Gpc-B1*, is located within an approximately 0.3-cM interval of *gwm508* and *gwm193* (Khan *et al*., 2000; Olmos *et al*., 2003; Distelfeld *et al*., 2004), or within 245-kb physical contig interval of *Xuhw89* and *Xucw71* (Distelfeld *et al*., 2006). Our *QGpc.usw-B5* QTL (Figure 3.3) was localized very close to *Gpc-B1* on chromosome 6BS (Khan *et al*., 2000; Olmos *et al*., 2003; Distelfeld *et al*., 2004), but was associated with 1000-kernel weight in half the environments (Table 3.7) and only associated with GPC QTL in one of the six environments. Thus this QTL is likely different from *Gpc-B1*.

At a physiological level, GPC is influenced by a number of factors including nitrogen uptake, assimilation, and remobilization to the grain during grain filling. The early steps of nitrate and ammonia assimilation or remobilization involve three gene families, those coding for nitrate reductase (*NAR*), nitrite reductase (*NIR*), glutamate synthase (*GOGAT*) (Boisson *et al*., 2005). The wheat glutamine synthetase (*GS2*) gene has been mapped to the telomeric regions of the group 2 chromosomes (Habash *et al*., 2007) and is likely not associated with the *QGpc.usw-B3* QTL on 2B in this study which is located near the centromere (Figure 3.3). Using Chinese Spring chromosome deletion lines, Fd-Glutamate synthase (*Fd-GOGAT*) genes have been localized to the group 2 chromosomes near the centromere (Boisson *et al*., 2005) and maybe associated with the *QGpc.usw-B3* identified in this study. A nitrate reductase (*NAR*) gene has been reported on the short arm of chromosome 7A (Habash *et al*., 2007), and thus would not be associated with *QGpc.usw-A3* localized to the long arm of 7A.

The GPC QTL *QGpc.usw-A1* on chromosome 1A (Figure 3.3) localized to a region known to house gliadin and glutenin genes. The *Gli-A1* locus identified by Elouafi and Nachit (2004) was linked to *gwm136*, as was the *QGpc.usw-A1* QTL of this study. Protein quality was not evaluated in this study, but the influence of the 1A region on gluten strength needs to be assessed prior to recommending this QTL for marker assisted selection. An additional QTL on chromosome 1B (*QGpc.usw-B1*) was flanked by DArT markers *378083* and *344673* located in the distal region away from the centromere. The distal region of chromosome 1BS contains the *Gli-B1/Glu-B3 loci*

(Blanco *et al*., 1998) and is likely not associated with *QGpc.usw-B2*, as this QTL has a pleiotropic effect on 1000-kernel weight and yield in some environments (Table 3.7), and it is more probable that genetic factors influencing kernel size and/or yield are located in this region.

The GPC QTL *QGpc.usw-A3* identified in this population has yet to be reported in durum wheat, but is likely the same as that reported in the hexaploid wheat Avalon x Hobbit RIL population, as both are closely linked to *barc108* (Turner *et al*., 2004). The presence of a common QTL in durum and bread wheat confirms the importance of this QTL and suggests a common genetic mechanism for grain protein accumulation or remobilization in the two species. The *QGpc.usw-B3* marked by *wmc41* has yet to be reported, but it is homeologous to QTL identified on chromosome 2D which is also associated with *wmc41* (Prasad *et al*., 1999). The *wmc41* marker has been validated to be a good marker for high GPC QTL in hexaploid wheat (Harjit-Singh *et al*., 2001). Unfortunately, *wmc41* was not polymorphic in the 9370-DJ**3 x Strongfield validation population, and therefore could not be validated. However, 9370-DJ**3 had similar GPC to Strongfield in most environments (Table 3.8), perhaps because this line is already fixed for this major QTL.

The GPC QTL *QGpc.usw-A2* on 2A reported in this study was detected at three locations with no negative pleiotropic effect on 1000-kernel weight. However, this QTL was associated with reduced grain yield at SC 2002 and RG 2003. This QTL is likely similar to that reported for gluten strength in durum wheat DH population derived from the cross Kyle2*/Biodur (Knox *et al*., 2004). The linked marker *gwm339* reported in Kyle2*/Biodur population was also found to be closely linked to *QGpc.usw-A2* in population studied here. The same QTL was also reported in a RIL population of *T. turgidum* var. *durum* x *T. turgidum* var. *dicoccoides* which explained approx. 16.8% of phenotypic variation (Blanco *et al*., 2006). Thus, our results also confirm the presence of GPC QTL on chromosome 2A marked by *gwm339*.

The most conservative approach to justify QTL useful for marker assisted selection is by selecting those QTL that are identified in multiple environments and/or in multiple populations, and are not associated with pleiotropic effects on other important traits. In this study, several QTL were identified in the DT695 x Strongfield population,

but the two stable QTL, *QGpc.usw-B3* on 2B and *QGpc.usw-A3* 7A, are the most promising targets for marker assisted breeding because there were consistently expressed in a range of environments (Table 3.6) representing dramatically different conditions (Table 3.1). Individually, *QGpc.usw-B3* and *QGpc.usw-A3* result in an average QTL effect of 0.23 and 0.30%, respectively, with no negative pleiotropic effect on 1000-kernel weight. However, these two QTL were associated with a small reduction in yield in some environments. In the DT695 x Strongfield population *QGpc.usw-A3* was significantly associated with GPC in all environments (Table 3.6), but the high GPC allele at this QTL was only associated with reduced yield at RG 2003 (Table 3.7). Similarly, in the validation population, this QTL was associated with GPC in all five environments (Table 3.10), but only associated with reduced yield in two environments. Despite the association with reduced yield at these sites, a high proportion of lines (>58%) had yield equal to or greater than Strongfield, coupled with high GPC equivalent to Strongfield at these two sites (Table 3.10). This, together with the strong negative correlation between yield and GPC suggests that this QTL is only weakly associated with grain yield.

The discovery of molecular markers linked to phenotypic variation is only a preliminary step in establishing a marker-assisted selection program for genetic improvement as QTL may be population-specific and their effects on phenotypic expression may be significantly overestimated, particularly for complex traits like GPC. In this study, we have validated the potential of selecting for the *QGpc.usw-A3* QTL in a separate validation population as this QTL was expressed in the DT695 x Strongfield population in the majority of environments evaluated. Averaged over five locations, selection for the *QGpc.usw-A3* QTL independently resulted in an average 0.3% increase in protein. This result confirms that selection of this locus, at least in crosses involving Strongfield, can be an effective means to improve or maintain GPC levels in durum wheat breeding programs. Given the consistent expression of the *QGpc.usw-A3* QTL in a number of locations and in two populations, this QTL should be a useful target for marker assisted selection. However, validation of this QTL in diverse genetic backgrounds is still required to confirm that this marker would be useful in other breeding populations. Production of near isogenic lines (NILs) for the *QGpc.usw-A3*

QTL in multiple genetic backgrounds should be examined to confirm the expression of this QTL in those backgrounds. Confirmation would further warrant efforts to pursue finer mapping and positional cloning using the NILs to elucidate the gene(s) associated with elevated GPC at this QTL. In addition, these NILs could be used to better understand the physiological mechanisms associated with elevated GPC as the result of this QTL.

3.6. Conclusion

In this study, QTL associated with variation for GPC were identified on the homoeologous group chromosomes 1, 2, and 7 and on chromosome 5B and 6B in the DT695 x Strongfield population, but QTL x environment interaction was evident. The two most stable QTL *QGpc.usw-B3* and *QGpc.usw-A3* were consistently expressed in a range of environments representing dramatically different climatic conditions. Positive alleles for GPC at these loci were contributed by the high-GPC parent Strongfield. Individually, the *QGpc.usw-B3* and *QGpc.usw-A3* QTL resulted in an average GPC effect of 0.23% and 0.30%, respectively, with no negative pleiotropic effect on kernel weight. These two QTL were associated with a small reduction in yield in some environments. The *QGpc.usw-A2* on chromosome 2A that was contributed by DT695 might also worth considering as this QTL and its marker *gwm339* was reported previously in a DT695-related DH population. This QTL contributed an average allele effect of 0.27% for GPC. The close association between *barc108* and the *QGpc.usw-A3* QTL was validated in another Strongfield-related DH population. Depending on environments, selection for the Strongfield allele at *barc108* resulted in +0.4% to +1.0% increase in GPC. Given the consistent expression of the *QGpc.usw-A3* QTL in diverse genetic backgrounds and in a number of locations, this QTL should be a useful target for marker assisted selection. In conclusion we identified a major QTL for high protein concentration originating in domesticated durum from the cultivar Strongfield that could aid durum wheat breeders in selecting for this important trait at earlier generations.

Chapter 4

PHYSIOLOGICAL ANALYSIS OF GRAIN NITROGEN ACCUMULATION IN DURUM WHEAT

4.1. Abstract

Grain protein concentration is influenced by nitrogen (N) uptake, assimilation, and remobilization to the grain. Little is known about these physiological factors in relation to variable expression of GPC in durum wheat grain. The objective of this research was to investigate N remobilization and post-anthesis N uptake (NUP) in relation to GPC. Eighteen genotypes varying in GPC were grown at three pre-selected locations with varying levels of soil N. Significant genotype x environment (GxE) interactions were detected for 1000-kernel weight (g), grain yield (kg ha^{-1}), GPC, grain protein yield (kg ha^{-1}) and post-anthesis N uptake (mg $plant^{-1}$), but not for remobilized N (NR; mg $plant^{-1}$). Irrespective of soil N levels, N remobilization was the major contributor to grain N, accounting for an average of 84.3% of grain protein. This study confirmed that introgression of chromosome 6B from *T. durum* var. *dicoccoides* into Langdon resulted in increased GPC, and this GPC increase was due to higher N remobilization. There was variation in post anthesis NUP among the cultivars tested, and Strongfield and a series of doubled haploid lines pre-selected for high GPC from the cross DT695 x Strongfield showed more post-anthesis NUP. The semi-dwarf cultivars Commander and Westbred 881 had low GPC, and because they did not accumulate N post-anthesis, most grain protein was derived only from N remobilization.

4.2. Introduction

At a physiological level, grain protein formation involves several steps, including accumulation of N in vegetative organs and its subsequent remobilization to reproductive organs for protein synthesis (Norman *et al*., 1992; Sheehy *et al*., 2004; Zhu *et al*., 2007). These processes are under genetic control, but their expression is also influenced by growing environments and their interactions with genotypes (Triboï *et al*., 2000). During vegetative growth, plants absorb N in the form of either nitrate or

ammonium ions from the soil to meet the needs of structural growth. In the plant, N is further converted to ammonia, and is rapidly incorporated into organic compounds through a number of metabolic pathways (Barker and Bryson, 2007). The early steps of nitrate and ammonia assimilation or remobilization involve three gene families, those coding for nitrate reductase (*NAR*), nitrite reductase (*NIR*), glutamine synthetase/glutamate synthase (*GS/GOGAT*) (Good *et al*., 2004; Boisson *et al*., 2005).

During the grain filling period, the amount of N uptake is much smaller than the demand for N accumulation in grains. As such, a large part of N is remobilized from the vegetative organs in the form of amino acids to the grains for protein synthesis (Mae, 1997; Zhu *et al*., 2007). The proportion of remobilized N in the harvested grain is environmentally-dependent and can account for 60 to 92% of the total grain protein (Austin *et al*., 1977; Cox *et al*., 1985a, 1985b, 1986; Papakosta and Garianas, 1991). Uptake of nitrate and ammonia from the soil after flowering also tended to elevate GPC (Blackman and Payne, 1987), especially under the condition of adequate soil N and water availability (Egle *et al*., 2007). In winter wheat, both N remobilization and post-anthesis NUP contribute to elevated GPC, and NAR activity is highly correlated with post-anthesis NUP and GPC (Kichey *et al*., 2007).

In general, greater vegetative N concentration at anthesis results in a larger pool of available N for translocation to grains, and consequently higher GPC (Ntanos and Koutroubas, 2002). Wang *et al*. (2003) reported that Canadian bread wheat cultivars with elevated GPC were more efficient at translocating N from vegetative tissue to the grains. Elevated GPC in the durum wheat introgression line Langdon(DIC-6B) was also due to better N remobilization to the grains (Deckard *et al*., 1996; Kade *et al*., 2005). However, there are currently no studies on the physiology of N accumulation in current durum wheat cultivars showing variable expression of GPC. In particular, expression of high GPC is poor in semi-dwarf durum wheat cultivars (McClung *et al*., 1986; Pinthus and Gale, 1990), and understanding the physiological basis for this deficiency could allow durum wheat breeders to develop a strategy to elevate GPC in semi-dwarf types.

The objective of this research was to investigate N remobilization and post-anthesis NUP in relation to elevated GPC in a collection of durum wheat cultivars and breeding lines with variable GPC.

4.3. Materials and Methods

4.3.1. Plant materials and experimental design

Eighteen genotypes varying in GPC were used in this study. These genotypes included Strongfield (high GPC) and DT695 (low GPC), the isogenic pair Langdon(DIC-6B) and Langdon, semi-dwarf cultivars Commander and Westbred 881, and two groups of doubled haploid (DH) selections derived from the cross DT695 x Strongfield (see Chapter 3). Based on 2002 and 2003 field trials, these DH lines did not show significant differences ($P>0.05$) in grain yield, days to maturity, or seed size, but were variable for GPC.

Experiments were carried out at Crop Research Farms of the University of Saskatchewan, Canada (Kernen in 2005, and at Goodale and Nasser in 2006). A randomized complete block design with three replicates was used for each environment. Prior to planting, soil N levels at all experimental locations were determined at 0 to15-, 15 to 30- and 30 to 60-cm depths. Soil samples were homogenized and analyzed for NO_3-N content at the Enviro-Test Laboratories Agricultural Services, Saskatoon, Saskatchewan, Canada. Plot size for each experimental unit was 1.2 x 3.6 m^2 consisting of five rows with a 20 cm between-row space. The seeding rate was 250 seeds per m^{-2}. Seeding was conducted on May 14th 2005, May 18th, May 23rd, 2006 at Kernen, Goodale and Nasser, respectively. An 11-55-0 (N-P-K) fertilizer was applied at the rate of 56.7 kg $hectare^{-1}$. No irrigation was supplied at any of the environments and monthly precipitation (mm $month^{-1}$) during the growing seasons was recorded (Table 4.2).

4.3.2. Trait evaluation

For each plot, plants in the first row were used for tissue sampling, while plants in the remaining four rows were used for plot-based evaluation of grain yield (kg ha^{-1}), days to heading (59 of the Zadocks growth scale; ZGS), days to maturity (90 ZGS), plant height (cm), GPC (%), and grain protein yield (kg ha^{-1}; grain yield x GPC). For tissue N-analysis, five plants were randomly taken on each of four sampling dates: at anthesis (65 ZGS); Zadocks *et al*., 1974), milk stage (75 ZGS), dough stage (85 ZGS) and physiological maturity (90 ZGS). Samples were partitioned into lower leaves (all leaf blades except the flag leaf), stem (culm and leaf sheath), flag leaf, and spike (chaff

and grain), and dried at 60^0C for 72 hours. The dried samples were weighed and then ground using a Thomas Willey laboratory grinder (model 4, Arthur H. Thomas Co., Philadelphia, PA) fitted with a 1 mm^2 screen, and N content was determined using the combustion method with a FP-528 LECO N Analyzer (LECO Corporation, St Joseph, MI). Protein concentration was determined as N concentration multiplied by a coefficient of 5.7 (Williams *et al*., 1998; AACC, 2000). Nitrogen content (mg) was calculated as dry weight of the respective plant part multiplied by its N concentration. Samples were measured for moisture using AACC approved method No. 44-15A (AACC, 2000), and protein concentration (%) and N content (mg) are presented on a 14% moisture basis.

Total plant nitrogen (TPN; mg $plant^{-1}$) was determined by summing the N content (mg) of all individual plant parts. The remobilized N (NR; mg $plant^{-1}$) was calculated as TPN at anthesis not recovered from vegetative tissue at physiological maturity. Post-anthesis NUP (mg $plant^{-1}$) was calculated as the difference between TPN at physiological maturity and TPN at anthesis. The proportion of the remobilized N in the grain (GrainNR) was calculated as the ratio of NR to the total grain N at physiological maturity. Vegetative N (VegN) was calculated as the sum of N content (mg) of all individual vegetative parts. Nitrogen harvest index (NHI) was calculated as the ratio between grain N to the TPN at physiological maturity. All data related to N content are presented on a per plant basis.

4.3.3. Statistical analysis

Data from all locations were analyzed using PROC MIXED of SAS (SAS Institute Inc., 2003). Because genotypes and environments were intentionally pre-selected, these factors were treated as fixed effects. Least square (LS) means were estimated and the standard error of differences (SED) was estimated using the "pdiff" command.

4.3.4. *Barc108* marker analysis

The microsatellite marker *barc108* that was associated with the GPC QTL *QGpc.usw-A3* on chromosome 7A (Chapter 3) was used to genotype the DH selections

(after selections were made). The DNA extraction and PCR reaction were identical to those in section 3.3.3. Contrast analysis was also carried out for post-anthesis NUP, NR, NHI and GrainNR for DH selections carrying Strongfield allele at *barc108* versus DH selections DT695 allele at *barc108*.

4.4. Results

4.4.1. Environmental conditions

The Kernen, Goodale and Nasser crop research farms are located in the black soil climatic zone of Saskatchewan with clay, loam, and clay loam textures, respectively. A pre-seeding soil analysis indicated variable soil N levels (Table 4.1). Soil N levels were highest at Kernen with 34.6 kg ha^{-1} at 0-15 cm depth. Goodale had lower soil N than Nasser at the 0-15 cm depth, but more N at the 15-20 cm and 30-60 cm depths (Table 4.1).

Above average precipitation was received during the early growing season in May at Goodale and Nasser, but May precipitation was lower than the 30-year average at Kernen (Table 4.2). Above average precipitation was received during vegetative growth in June at all environments. In contrast, lower than average precipitation was received at Goodale and Nasser in 2006 during the flowering period in July, with average precipitation at Kernen. Average precipitation was received during the grain fill period in August at Goodale and Nasser, but above average precipitation at Kernen (Table 4.2).

Table 4.1. Soil NO_3-N level based on pre-seeding soil test report (Enviro-Test Laboratories Agricultural Services, Saskatoon, Canada) at the experimental locations of Kernen 2005, Goodale 2006 and Nasser 2006.

	NO_3-N level (kg ha^{-1})		
	Kernen 2005	Goodale 2006	Nasser 2006
0 to15 cm-depth	34.6	13.6	17.3
15 to 30 cm-depth	13.6	19.6	13.6
30 to 60 cm-depth	45.7	32.1	19.8
Total	93.9	65.3	50.7

Table 4.2. Precipitation (mm month^{-1}) during the study at the experimental locations of Kernen 2005, Goodale 2006 and Nasser 2006. Average precipitation and monthly temperature at each environment were collected from Kernen research station metrological data.

	Precipitation (mm month^{-1})					
	May	June	July	August	Sept	Total
Kernen 2005	31	193	53	54	74	405
Goodale 2006	59	113	37	37	144	390
Nasser 2006	57	102	39	35	46	279
Saskatoon 30-year (1975-2005) monthly average **)	42	61	57	35	29	224

**) adapted from http://www.worldweather.org/056/c00628.htm

4.4.2. Effect of environments on agronomic traits, TPN and its partitioning, and N remobilization and post-anthesis N uptake related traits

Significant environmental effects were obvious for all measured variables including plant height, days to heading, days to maturity, 1000-kernel weight (Table 4.3; Appendix 4.1), grain yield, GPC and grain protein yield (Table 4.4; Appendix 4.1). Averaged over all cultivars, plant height and 1000-kernel weight were significantly lower ($P<0.05$) at Nasser, but there was no difference between Kernen and Goodale (Table 4.3). On average, cultivars headed six days later at Kernen than at Goodale and Nasser, and differences ($P<0.01$) in days to maturity were significant among all three environments. As expected, grain yield was highest at Kernen and lowest at Nasser (Table 4.4). Grain protein concentration (%) and grain protein yield (kg ha^{-1}) were also highest at Kernen.

Table 4.3. Least square means of plant height, days to heading, days to maturity and 1000-kernel weight at at Kernen 2005, Goodale 2006 and Nasser 2006.

Locations	**Plant height (cm)**	**Days to heading (d)**	**Days to maturity (d)**	**1000-kernel weight (g)**
Kernen 2005	115	60	108	44.7
Goodale 2006	110	54	91	45.4
Nasser 2006	80	54	85	39.6
LSD ($P<0.05$)	4	1	1	1.2

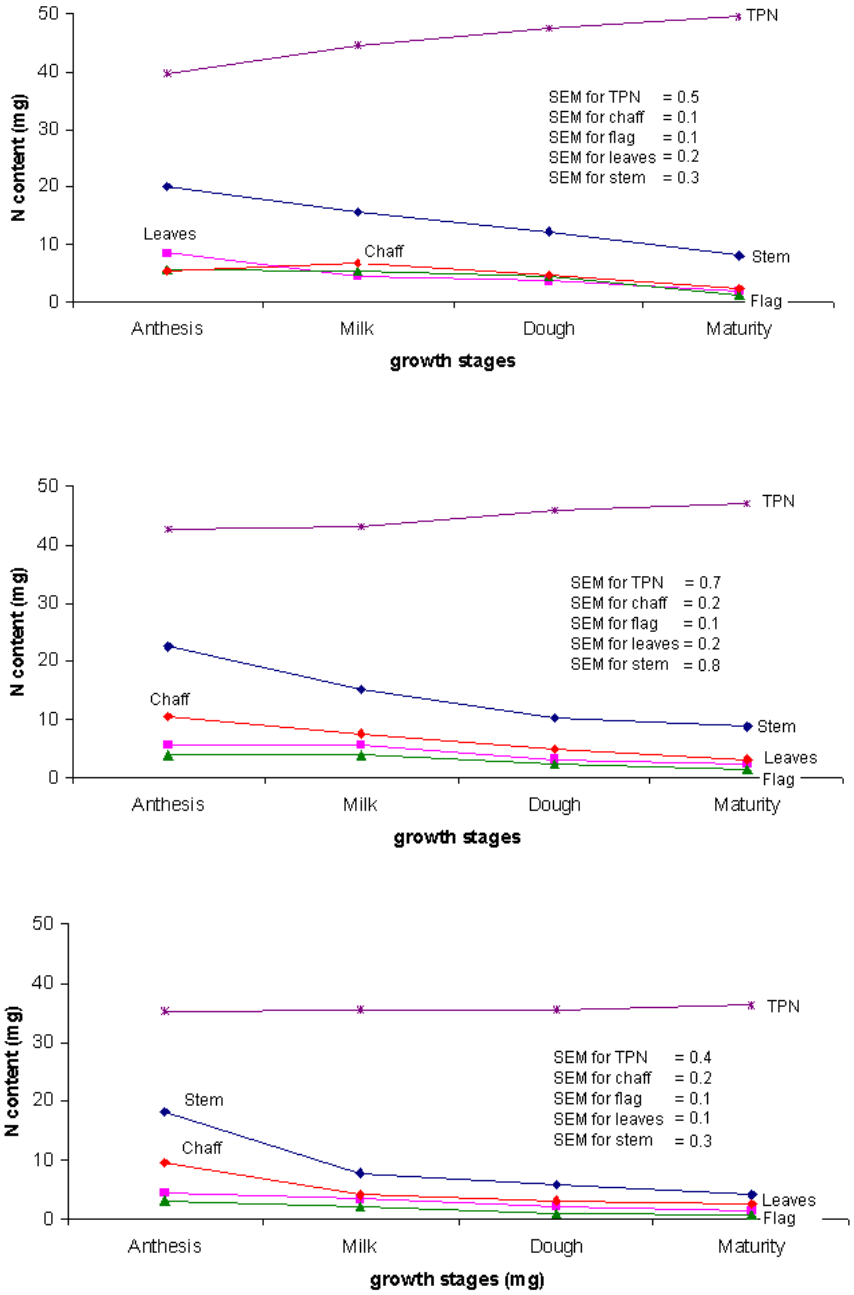

Figure 4.1. Changes in total plant nitrogen (TPN; mg plant^{-1}), stem, leaves, flag leaf, and chaff portions at Kernen 2005 (top), Goodale 2006 (middle) and Nasser 2006 (bottom). Each point in the graph line represents data of 18 genotypes with three replicates. SEM = standard error of means.

Table 4.4. Least square means of grain yield, GPC and protein yield at Kernen 2005, Goodale 2006 and Nasser 2006.

Locations	Grain yield (kg ha^{-1})	GPC (%)	Protein yield (kg ha^{-1})
Kernen 2005	5518	13.7	757
Goodale 2006	3884	11.9	462
Nasser 2006	2355	10.2	239
LSD (P<0.05)	137	0.2	20

Averaged over all cultivars evaluated, variation in total plant nitrogen (TPN) was detected among environments throughout phenological development. Total plant nitrogen increased over sampling time, but the rates of increase were different among locations (Figure 4.1; Appendix 4.2 and 4.3). The TPN increase at Kernen was the greatest, followed by Goodale, while no significant TPN increase was observed after anthesis at Nasser (Appendix 4.1, and 4.3). Significant differences in the total accumulated N at anthesis (TPN-An) were also detected among environments with Nasser having the lowest TPN-AN (Figure 4.1; Appendix 4.3). At all sampling times, the stem (culm and leaf sheath) contained the majority of vegetative N, and was the predominant source of N for remobilization (Figure 4.1). Following anthesis, N content decreased in almost all vegetative organs in all three environments, with the most rapid decrease observed at Nasser, but increase in N content was still detected in the chaff and flag leaf at Kernen, and delay in N loss was observed in the chaff and flag leaf at Goodale (Figure 4.1).

Averaged over all cultivars, grain nitrogen (GN), NR and post-anthesis NUP varied among the contrasting environments (Figure 4.2; Appendix 4.2 and 4.3). At Kernen and Goodale, there was a linear increase in GN after anthesis, but at Nasser, the rate of increase declined after the milk stage. Post-anthesis NUP was only evident at Kernen and Goodale.

At physiological maturity, the amount of the remobilized N (mg plant^{-1}) was constant across environments but differences in nitrogen harvest index (NHI), and the proportion of grain N derived from remobilization (GrainNR) were evident (Table 4.5). Post-anthesis NUP was highest at Kernen, followed by Goodale and Nasser. At Nasser, most of N in the grains was derived from N remobilization (Table 4.5).

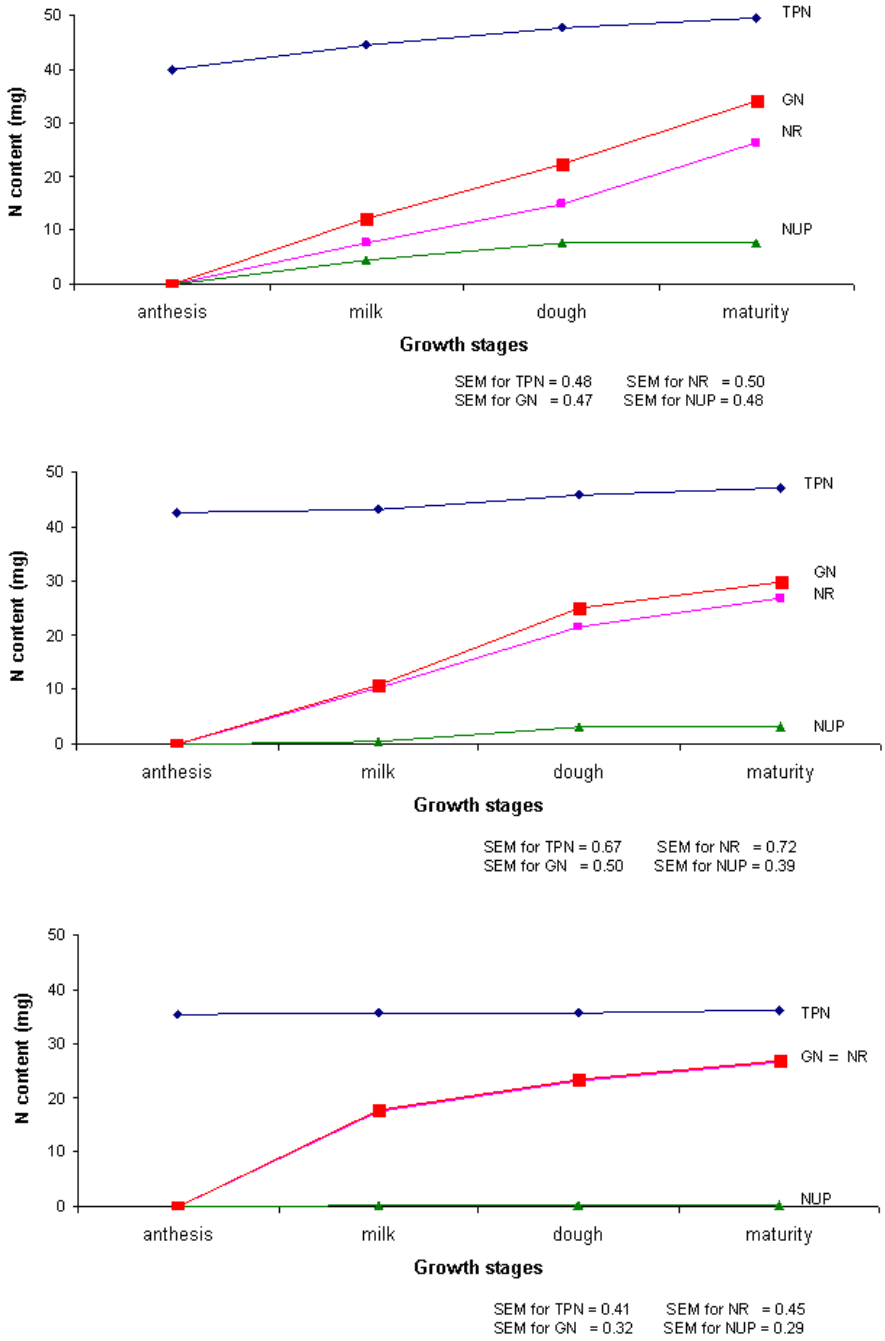

Figure 4.2. Changes in total plant nitrogen (TPN; mg plant^{-1}) in relation to grain nitrogen (GN; mg plant^{-1}), remobilized N (NR; mg plant^{-1}) and post-anthesis NUP (mg plant^{-1}) at four sampling times during grain fill at Kernen 2005 (top), Goodale 2006 (middle) and Nasser 2006 (bottom). Each point represents the data of 18 genotypes with three replicates. SEM = standard error of means.

Table 4.5. Least square means of nitrogen harvest index (NHI), remobilized N (NR), post-anthesis NUP, and the proportion of the remobilized nitrogen in the grains (Grain NR) at Kernen 2005, Goodale 2006 and Nasser 2006.

Locations	NHI (%)	NR (mg)	Post-anthesis NUP (mg)	GrainNR (%)
Kernen 2005	72.3	26.2	9.7	73.6
Goodale 2006	76.6	26.6	4.6	85.7
Nasser 2006	75.7	26.9	1.9	93.5
LSD (P<0.05)	3.4	1.9	1.0	2.8

4.4.3. Effect of genotypes, and GxE interaction on agronomic traits, TPN, and N remobilization and post-anthesis N uptake related traits

Significant differences among genotypes were observed for plant height, days to heading, and days to maturity at each environment, but the combined analysis of variance revealed that the GxE interaction was not significant (P>0.05) (Appendix 4.1). As expected, Strongfield and DT695 were taller than the semi-dwarf cultivars Commander and Westbred 881, while the isogenic pair Langdon(DIC-6B) and Langdon had similar height, but were the tallest cultivars (Table 4.6). Days to heading and maturity were also similar for Langdon and Langdon(DIC-6B) in all environments. Contrast analysis revealed that days to heading and days to maturity were similar between the high- and low-GPC DH selections (P>0.05), except for days to maturity at Goodale, where the low-GPC DH selections matured on average six days earlier than the high-GPC DH selections (Table 4.6). Plant heights were similar among the majority of high- and low-GPC DH selections, except for the low-GPC DAQ-02* which was significantly taller than all other DH selections in all environments (Table 4.6).

The GxE interaction was significant (P<0.01) for 1000-kernel weight and grain yield (Appendix 4.1). Significant variation among genotypes for 1000-kernel weight was observed at Kernen and Goodale, but not at Nasser. At Kernen, where the largest range in seed size was observed, Langdon had significantly smaller seeds than Langdon(DIC-6B) (Table 4.7). Strongfield was statistically higher yielding (P<0.01) than the other check cultivars at Kernen but expressed grain yield similar to DT695 and Commander at Goodale and Nasser (Table 4.7). Westbred 881 was consistently the lowest yielding cultivar at all sites. No statistical differences (P>0.05) in grain yield

were detected between Langdon(DIC-6B) and Langdon at any of the environments. The range in yield among the high- and low-GPC DH selections was similar at Kernen and Goodale (Table 4.7). Averaged over genotypes within each selection group, the high-GPC DH selections yielded less grain ($P<0.05$) than the low-GPC DH selections only at Nasser (Table 4.7).

Significant variation in GPC was detected among genotypes and growing conditions (Appendix 4.1). Averaged over all three environments, Langdon(DIC-6B), which carries *Gpc-B1,* consistently had 2.2% higher GPC than Langdon (Table 4.7). Strongfield had significantly higher GPC than DT695 only at Nasser, but higher GPC than the semi-dwarf cultivar Commander at Kernen and Goodale. Westbred 881 expressed GPC similar to Strongfield and Langdon(DIC-6B) in all environments (Table 4.7). The high-GPC DH selections had higher GPC ($P<0.01$) than the low-GP DH selections in all environments (Table 4.7). Averaged over all three environments, the difference in GPC between these two selected groups was 1.2%. However, at Goodale, some lines in the low-GPC DH selection group had GPC similar to the average of the high-GPC DH selections. Likewise, DAC-04*, a high-GPC DH selection had GPC similar to most of the low-GPC DH selections at that environment (Table 4.7).

Consistent with GPC and grain yield, grain protein yield also varied among genotypes and environments, and the GxE interaction was significant (Table 4.7; Appendix 4.1). Strongfield was the only check cultivar that expressed high grain yield and high GPC, and had the highest grain protein yield (Table 4.7). All high-GPC DH selections had grain protein yields similar to Strongfield in all environments, except DAC-04*, which had low yield, and thus lower grain protein yield. Compared to Langdon, Langdon(DIC-6B) had higher grain protein yield at Kernen ($P<0.05$), but not at Goodale and Nasser (Table 4.7).

Total plant nitrogen at anthesis (TPN-An) describes the capacity of the plant to accumulate and store N in vegetative organs prior to remobilization to the developing grains. At Kernen and Goodale, Langdon had the lowest TPN-An of the check cultivars, and was significantly lower than its near isogenic pair Langdon(DIC-6B) (Table 4.8). No significant differences in TPN-An were detected among genotypes at Nasser.

Table 4.6. Least square means of plant height, heading date and maturity date of the evaluated genotypes at Kernen 2005 (Krn), Goodale 2006 (Gdl) and Nasser 2006 (Nsr).

Genotypes	Plant height (cm)				Heading Date [d.a.s. a)]				Maturity date [d.a.s. a)]			
	Krn	Gdl	Nsr	Mean	Krn	Gdl	Nsr	Mean	Krn	Gdl	Nsr	Mean
Check cultivars												
Strongfield	108	103	79	97	60	52	53	55	108	90	85	94
DT695	121	113	81	105	59	53	53	55	108	91	85	95
Commander	94	92	70	86	59	53	54	55	106	91	86	94
Westbred 881	82	82	71	78	56	51	51	53	104	90	84	93
Langdon(DIC-6B)	135	141	98	124	60	54	54	56	106	89	96	94
Langdon	134	138	98	123	60	54	55	56	110	91	85	95
Low-GPC DH												
DAH-46*	106	102	76	95	60	54	55	56	108	85	85	95
DAN-08*	118	113	79	103	62	55	55	57	108	85	85	95
DAE-01*	123	113	83	106	60	54	54	56	110	85	85	95
DAQ-02*	133	126	95	117	62	55	55	57	110	86	86	96
DAD-04*	115	110	76	101	62	55	55	57	109	86	86	96
DAH-07*	113	110	78	100	61	53	53	56	109	84	84	95
Average	**118**	**116**	**81**	**103**	**61**	**54**	**54**	**56**	**109**	**85**	**85**	**95**
High-GPC DH												
DAD-09*	124	116	85	108	59	53	52	55	108	91	84	94
DAB-06*	101	91	71	88	60	53	53	55	109	91	86	95
DAG-02*	123	115	82	107	61	54	53	56	111	92	86	96
DAD-10*	110	103	73	95	60	53	54	56	109	91	86	95
DAC-04*	114	106	73	99	59	52	54	55	107	91	85	94
DAL-08*	111	102	77	95	61	53	53	56	110	90	86	96
Average	**114**	**105**	**77**	**99**	**60**	**53**	**53**	**56**	**109**	**91**	**86**	**95**
L vs H contrast[a]	**ns**	******	**ns**	*****	**ns**	**ns**	**ns**	**ns**	**ns**	******	**ns**	**ns**
LSD (P<0.05)	**5.5**	**7.2**	**6.7**	**3.7**	**1**	**1**	**1**	**0.5**	**2**	**2**	**2**	**1**

[a]Contrast analysis of means of high and low GPC DH selections where ns = not significant (p>0.05), * significant at P<0.05 and ** significant at P<0.01.

Table 4.7. Least square means of 1000-kernel weight, grain yield, GPC and protein yield of the evaluated genotypes at Kernen 2005 (Krn), Goodale 2006 (Gdl) and Nasser 2006 (Nsr).

Genotypes	1000-kernel weight (g)				Grain yield (kg ha^{-1})				GPC (%)				Protein yield (kg ha^{-1})			
	Kern	Gdl	Nsr	Mean	Krn	Gdl	Nsr	Mean	Krn	Gdl	Nsr	Mean	Krn	Gdl	Nsr	Mean
Check cultivars																
Strongfield	44.4	45.3	38.2	42.6	6117	4130	2484	4244	13.8	13.7	10.9	12.8	846	565	269	560
DT695	47.3	49.0	41.1	45.9	5438	4389	2564	4130	13.4	12.7	9.2	11.7	728	555	236	507
Commander	47.0	49.7	41.6	46.1	5785	4082	2460	4109	13.0	11.5	10.2	11.6	755	470	251	492
Westbred 881	44.6	45.7	42.9	44.4	4505	3529	2048	3361	14.0	12.9	12.2	13.0	631	455	250	445
Langdon(DIC-6B)	48.9	44.5	35.7	43.0	4964	3280	1614	3286	15.8	11.6	12.0	13.1	784	382	195	454
Langdon	41.6	43.5	38.5	41.2	4713	2838	1895	3149	12.2	10.7	9.9	10.9	578	301	187	355
Low-GPC DH																
DAH-46*	44.9	44.9	37.6	42.5	6039	3658	2621	4106	12.4	11.2	9.0	10.9	751	406	236	464
DAN-08*	44.3	45.8	42.1	44.1	6254	3717	2503	4158	12.4	11.0	9.8	11.1	773	408	245	475
DAE-01*	40.9	45.1	39.3	41.8	5606	3745	2646	3999	14.0	10.6	9.6	11.4	784	398	254	479
DAQ-02*	46.5	45.6	40.9	44.3	5172	3583	2563	3773	13.1	12.5	9.2	11.6	679	445	234	453
DAD-04*	38.9	42.1	35.3	38.8	5912	4456	2578	4315	12.8	11.0	9.0	10.9	758	490	233	494
DAH-07*	51.2	47.0	40.5	46.2	5038	3993	2684	4205	12.6	12.2	9.7	11.5	748	489	259	498
Average	**44.5**	**45.1**	**39.3**	**43**	**5670**	**3859**	**2599**	**4093**	**12.9**	**11.4**	**9.4**	**11.2**	**749**	**439**	**244**	**477**
High-GPC DH																
DAD-09*	41.7	42.8	40.4	41.6	5335	4272	2295	3967	14.7	12.4	10.2	12.4	783	530	234	516
DAB-06*	44.8	45.3	38.1	42.7	5592	4501	2171	4088	15.0	12.5	10.1	12.5	839	563	219	540
DAG-02*	46.9	48.0	39.1	44.7	5646	4165	2430	4080	14.6	12.8	10.2	12.2	824	486	247	519
DAD-10*	47.7	44.5	41.4	44.6	6075	3754	2297	4042	13.9	12.7	10.9	12.5	845	475	252	524
DAC-04*	42.4	44.1	40.6	42.4	4885	3682	2215	3594	14.9	11.0	11.2	12.4	726	410	249	462
DAL-08*	41.3	44.6	38.9	41.6	5345	4136	2318	3943	15.0	11.6	10.9	12.5	798	483	255	512
Average	**44.1**	**44.9**	**39.8**	**42.9**	**5480**	**4085**	**2288**	**3952**	**14.7**	**12.2**	**10.6**	**12.4**	**803**	**491**	**243**	**512**
L vs H contrast[a]	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	*****	**ns**	******	******	******	******	******	******	******	******
LSD (P<0.05)	**3.4**	**3.7**	**4.6**	**2.3**	**402**	**792**	**281**	**335**	**0.9**	**1.1**	**1.2**	**0.6**	**81**	**104**	**42**	**49**

[a]Contrast analysis of means of high and low GPC DH selections where ns = not significant (p>0.05), * significant at P<0.05 and ** significant at P<0.01.

Averaged over all environments, Strongfield had higher ($P<0.05$) TPN-An than all other check cultivars, but no differences were detected among the high- and low-GPC DH groups in any environment (Table 4.8). The remaining N in the vegetative organs at physiological maturity (VegN-PM) was similar among the check cultivars. However, in all three environments, VegN-PM was significantly higher in the high-GPC selections compared to the low-GPC DH selections at all environments (Table 4.8).

Corresponding with stem (culm and leaf sheath) as the primary source of vegetative N (Figure 4.1), variation in stem N content at anthesis was observed among the genotypes (Table 4.9). At Kernen and averaged over locations, the stem N content of Strongfield was similar ($P>0.05$) with that of DT695, but was significantly greater than the stem N content ($P<0.05$) of the semi-dwarf cultivars Commander and Westbread 881. A tendency that high GPC cultivars had higher stem N content was still observed at Goodale and Nasser. Averaged over locations, Langdon(DIC-6B) had higher stem N content than Langdon. Except at Nasser, low-GPC DH selections demonstrated higher stem N content than high-GPC DH selections (Table 4.9).

Remobilized N (mg plant^{-1}) was assessed as the TPN at anthesis not recovered from vegetative tissue at physiological maturity. Statistical differences in NR were detected among cultivars in all three environments. Strongfield showed higher NR than the other checks at Goodale and Nasser but no statistical difference between Langdon(DIC-6B) and Langdon was detected at Nasser, or between Strongfield and DT695 and the semi-dwarf cultivars (Table 4.10). At Goodale, Commander remobilized less N than Strongfield, and was more similar to DT695 at that environment. Westbred 881 had the lowest NR at Kernen and Nasser, but was statistically similar to Strongfield at Goodale. No significant differences in NR were detected between the high- and low-GPC DH selections, although there was a tendency for high GPC cultivars to demonstrate higher TPN-An (Table 4.8) and higher NR (Table 4.10).

Table 4.8. Least square means of total plant nitrogen at anthesis (TPN-An) and vegetative nitrogen at physiological maturity (VegN-PM) of the evaluated genotypes at Kernen 2005 (Krn), Goodale 2006 (Gdl) and Nasser 2006 (Nsr).

Genotypes	TPN-An (mg plant^{-1})				VegN-PM (mg plant^{-1})			
	Krn	Gdl	Nsr	Mean	Krn	Gdl	Nsr	Mean
Check cultivars								
Strongfield	39.8	43.3	36.7	39.9	12.6	14.3	8.3	11.7
DT695	39.6	40.0	32.5	37.4	13.8	17.6	8.3	12.7
Commander	38.1	39.8	33.5	37.1	13.0	16.3	9.1	12.8
Westbred 881	36.6	39.3	32.3	36.0	11.7	13.5	8.6	11.3
Langdon(DIC-6B)	41.5	39.8	36.9	41.5	12.8	12.8	8.0	11.2
Langdon	37.1	33.2	37.0	35.7	15.4	16.7	9.9	13.0
Low-GPC DH								
DAH-46*	40.8	43.7	34.8	40.4	12.2	11.9	8.2	11.9
DAN-08*	38.6	43.7	35.4	39.2	11.3	14.7	8.3	11.4
DAE-01*	46.1	44.5	38.4	43.0	22.5	19.2	10.6	17.4
DAQ-02*	41.0	44.7	35.9	40.5	12.0	15.2	9.2	12.1
DAD-04*	39.5	46.7	33.2	39.8	13.3	16.4	7.6	12.4
DAH-07*	41.7	46.6	34.8	41.0	12.9	16.4	8.7	12.6
Average	**41.3**	**45.0**	**35.4**	**40.7**	**14.1**	**15.6**	**8.8**	**12.9**
High-GPC DH								
DAD-09*	41.8	43.1	35.9	40.3	17.3	18.3	8.7	13.7
DAB-06*	42.5	45.0	38.2	41.9	22.0	25.2	9.0	16.2
DAG-02*	39.4	41.1	33.5	38.0	22.6	21.6	9.2	16.3
DAD-10*	39.1	43.1	34.2	38.8	17.0	22.6	8.8	14.3
DAC-04*	39.9	43.9	36.7	40.2	15.3	16.9	8.5	13.6
DAL-08*	37.9	43.9	37.8	39.9	21.8	20.9	18.3	15.7
Average	**40.1**	**43.4**	**36.1**	**39.9**	**19.3**	**20.9**	**10.4**	**15.0**
L vs H contrast[a]	**ns**	**ns**	**ns**	**ns**	******	******	*****	*****
LSD (P<0.05)	**3.1**	**5.0**	**5.5**	**2.3**	**3.1**	**4.0**	**1.4**	**1.5**

[a]Contrast analysis of means of high and low GPC DH selections where ns = not significant (P>0.05), * significant at P<0.05 and ** significant at P<0.01.

Table 4.9. Least square means of stem N content at anthesis of the evaluated genotypes at Kernen 2005, Goodale 2006 and Nasser 2006.

Genotypes	Stem N content (mg)			
	Kernen 2005	Goodale 2006	Nasser 2006	Mean
Check cultivars				
Strongfield	20.6	22.6	19.5	20.9
DT695	19.5	20.2	16.4	18.6
Commander	16.6	20.7	17.1	18.1
Westbred 881	18.0	20.2	16.3	18.1
Langdon(DIC-6B)	19.8	22.0	19.6	20.5
Langdon	20.4	16.3	19.6	18.8
Low GPC				
DAH-46*	19.0	24.1	18.9	20.7
DAN-08*	23.3	24.1	18.6	22.0
DAE-01*	24.1	23.5	17.8	21.8
DAQ-02*	21.7	24.3	21.0	22.3
DAD-04*	20.6	24.4	17.7	20.8
DAH-07*	19.9	28.2	18.0	22.1
Average	**21.4**	**24.8**	**18.7**	**21.7**
High GPC				
DAD-09*	22.0	23.6	19.5	21.7
DAB-06*	20.2	24.1	17.8	20.7
DAG-02*	19.6	19.0	17.2	18.6
DAD-10*	18.5	21.4	17.3	19.1
DAC-04*	20.9	24.4	17.4	20.9
DAL-08*	18.8	22.4	18.2	19.8
Average	**20.0**	**22.5**	**17.9**	**20.1**
L vs H contrast[a]	**	**	ns	**
LSD (P<0.05)	**2.5**	**4.2**	**3.9**	**2.3**

[a]Contrast analysis of means of high and low GPC DH selections where ns = not significant (P>0.05), * significant at P<0.05 and ** significant at P<0.01.

Statistical differences in post-anthesis NUP were detected among cultivars in all environments (Table 4.10; Appendix 4.1). Of the check cultivars, Strongfield had the highest post-anthesis NUP when data was averaged over all environments. At Kernen, Strongfield had significantly higher post-anthesis NUP than Commander and Langdon, but at Goodale, all of the check cultivars had similar post-anthesis NUP, except for Westbred 881 which had no detectable N uptake after anthesis. At Nasser, DT695 had similar post-anthesis NUP to Strongfield, but had better capacity for post-anthesis NUP than the semi-dwarf cultivars Commander and Westbred 881, and also better than Langdon and Langdon(DIC-6B). No significant difference ($P>0.05$) in post-anthesis NUP was detected between Langdon and Langdon(DIC-6B) in any of the environments, but post-anthesis NUP was numerically higher in Langdon(DIC-6B) at Kernen (Table 4.10). Variation was evident for post-anthesis NUP within the selected high- and low-GPC DH groups. DAE-01* had the highest post-anthesis NUP of the low-GPC DH selections and was statistically similar to that of the high-GPC DH selections when averaged over all environemtns. DAC-04*, the lowest yielding of the high-GPC DH selections (Table 4.7), expressed poor post-anthesis NUP relative to the remaining five high-GPC DH selections (Table 4.10) However, on average, the high-GPC selections showed greater post-anthesis NUP ($P<0.05$) at all three environments (Table 4.10).

At Nasser, NHI of high-GPC cultivars Strongfield and Langdon(DIC-6B) was significantly ($P<0.05$) higher than that of DT695 and Langdon, respectively (Table 4.10). The semi-dwarf cultivars Commander and Westbred 881, similar to DT695, had lower NHI. The low-GPC DH selections had similar NHI to the high-GPC DH selections in all environments ($P>0.05$), with most of low-GPC DH selections expressing NHI similar to Strongfield.

Nitrogen remobilization was the primary source of grain N for all cultivars, but the proportion of grain N derived from remobilization (GrainNR) was variable among cultivars in all environments (Table 4.10). At Kernen, Strongfield had the lowest GrainNR of the check cultivars, but was not statistically different from Langdon(DIC-6B) and DT695 (Table 4.10). Similarly at Nasser, the GrainNR for Strongfield was the lowest of all checks, but was not statistically different from DT695.

Table 4.10. Least square means of post-anthesis NUP, remobilized N (NR), nitrogen harvest index (NHI), and grain NR of the evaluated genotypes at Kernen 2005 (Krn), Goodale 2006 (Gdl) and Nasser 2006 (Nsr). The DH selections carrying Strongfield allele at *barc108* (+) and DT695 allele at *barc108* (-) are also presented.

Genotypes	NR (mg plant^{-1})				post-anthesis NUP (mg plant^{-1})				NHI (%)				GrainNR (%)			
	Krn	**Gdl**	**Nsr**	**Mean**	**Krn**	**Gdl**	**Nsr**	**Mean**	**Krn**	**Gdl**	**Nsr**	**Mean**	**Krn**	**Gdl**	**Nsr**	**Mean**
Check cultivars																
Strongfield	26.0	28.9	27.4	27.5	10.2	4.9	2.9	6.0	74.0	70.1	78.1	74.0	71.1	86.6	89.9	82.5
DT695	25.6	24.1	24.6	24.7	8.7	4.7	1.3	4.9	71.1	64.3	75.2	70.2	74.9	83.6	95.0	84.9
Commander	26.6	23.6	24.3	24.2	6.2	4.6	0.2	4.1	71.0	63.4	73.0	69.1	76.9	83.9	98.4	86.6
Westbred 881	22.2	26.1	23.6	24.7	8.3	0.0	0.9	2.7	72.0	66.6	74.0	70.9	81.8	95.1	95.6	91.2
Langdon(DIC-6B)	28.9	27.0	31.0	27.3	7.8	4.4	0.2	4.6	73.1	70.9	77.2	73.7	73.4	86.1	99.4	86.3
Langdon	22.4	19.5	27.0	23.0	4.8	5.3	0.5	3.6	65.1	64.5	72.4	67.3	84.1	78.2	97.9	86.1
Low-GPC DH[b]																
DAH-46* (+)	28.4	30.6	26.7	28.5	4.8	1.7	2.1	2.9	72.9	67.8	77.6	72.7	85.1	95.2	93.1	91.2
DAN-08* (-)	27.1	29.0	27.1	27.7	12.1	1.2	1.8	5.0	77.0	67.4	76.4	73.6	70.4	85.1	94.2	86.9
DAE-01* (-)	28.5	25.4	27.8	27.2	12.4	3.5	3.1	6.3	70.3	61.7	74.6	68.9	68.4	82.4	90.2	80.4
DAQ-02* (+)	28.9	29.6	26.7	28.4	7.1	6.8	1.7	5.2	74.8	69.8	74.6	73.7	80.8	84.5	94.1	86.5
DAD-04* (-)	25.9	30.3	25.5	27.2	8.9	0.8	1.2	3.6	72.0	65.0	75.5	70.8	74.6	97.3	94.9	88.9
DAH-07* (-)	28.5	30.3	26.1	28.3	8.4	3.2	3.3	5.0	73.7	67.8	77.3	72.9	77.3	88.3	85.3	83.6
Average	**27.9**	**29.2**	**26.7**	**27.9**	**9.0**	**2.9**	**2.2**	**4.7**	**73.5**	**66.6**	**76.0**	**72.1**	**76.1**	**88.8**	**92.0**	**86.3**
High-GPC DH																
DAD-09* (+)	28.3	28.1	27.2	27.9	9.9	5.1	3.5	6.1	74.0	68.6	76.4	73.0	73.8	84.8	89.5	82.7
DAB-06* (+)	27.4	27.1	29.2	27.9	15.1	9.2	3.2	9.2	79.8	67.1	78.1	73.0	64.6	74.8	91.0	76.8
DAG-02* (+)	24.0	24.1	26.7	24.1	13.6	7.9	3.4	8.0	70.8	67.6	74.3	70.2	63.8	75.2	90.9	76.6
DAD-10* (+)	26.2	25.9	25.4	25.8	13.2	11.4	4.7	8.1	75.3	67.3	75.1	72.6	66.6	74.1	93.8	78.1
DAC-04* (+)	26.2	27.0	28.0	26.9	8.5	4.6	1.0	3.9	71.0	64.0	76.7	70.5	76.9	91.4	96.5	88.3
DAL-08* (+)	22.2	28.0	28.4	26.2	14.3	4.7	2.8	6.9	69.9	67.2	75.7	70.9	61.1	85.1	94.4	80.2
Average	**25.7**	**26.7**	**27.5**	**26.5**	**12.4**	**7.2**	**3.1**	**7.0**	**73.5**	**67.0**	**76.1**	**71.7**	**67.8**	**80.9**	**92.7**	**80.5**
L vs H contrast[a]	**ns**	**ns**	**ns**	**ns**	******	******	**ns**	******	**ns**	**ns**	**ns**	**ns**	******	******	**ns**	*****
(+) vs (-) contrast[b]	**ns**	**ns**	**ns**	**ns**	**ns**	******	******	*****	**ns**	**ns**	**ns**	**ns**	**ns**	******	**ns**	**ns**
LSD (P<0.05)	**3.9**	**3.9**	**4.9**	**2.6**	**3.2**	**3.1**	**1.9**	**2.0**	**4.9**	**7.1**	**2.7**	**2.9**	**4.4**	**9.6**	**5.6**	**4.8**

[a]Contrast analysis of means of high and low GPC DH selections, [b]Contrast analysis of means of Strongfield allele at *barc108* (+) and DT695 allele at *barc108* (-).
ns = not significant (P>0.05), * significant at P<0.05 and ** significant at P<0.01. (+)Strongfield allele at *barc108*, (-)DT695 allele at *barc108*

At Goodale and Nasser, no significant difference in GrainNR could be detected between Langdon and Langdon(DIC-6B). At Goodale, no differences in GrainNR were noted among check cultivars, but the LSD was larger than observed at Kernen and Nasser (Table 4.10). Averaged over all three environments, the high-GPC DH selections had lower GrainNR than the low-GPC selections, but among environments, differences were only significant at Kernen and Goodale (Table 4.10).

4.4.4. *Barc108* marker analysis

Marker analysis showed that all the high-GPC DH selections carried the Strongfield allele at *barc108*, the molecular marker most closely associated with *QGpc.usw-A3* (the Strongfield allele that was associated with high GPC) (Table 4.10), the low-DH selections DAH-46* and DAQ-02* also carried the Strongfield allele at *barc108* allele (Table 4.10). Significant differences between DH selections carrying Strongfield allele at *barc108* (+) and DH selections carrying DT695 allele at *barc108* (-) in post-anthesis NUP were evident at Goodale, Nasser and average over all three environments, but not at Kernen. Differences between these two DH selections were not observed for NR, NHI and GrainNR at any environment, except for GrainNR at Goodale (Table 4.10).

4.5. Discussion

4.5.1. Effect of environments on agronomic traits, N remobilization and post-anthesis N uptake

Nitrogen is one of the building blocks of amino acids, proteins, and nucleic acids (Barneix *et al*., 2007), and is incorporated into cereal grains through two integrated processes over time, namely (a) uptake and assimilation of N available in the rhizosphere and (b) remobilization of previously acquired N in the vegetative biomass (Dalling, 1985; Cox *et al*., 1986; Weiland and Ta, 1992; Feller and Fischer, 1994; Dupont and Altenbach, 2003). As shown in this study, these two processes were environmentally-dependent, varied among genotypes, and were influenced by the amount of available N for uptake, and water availability. In addition, environmental effects on expression of TPN, VN and GN, and on N remobilization and post-anthesis NUP were evident throughout phenological development (Figure 4.1 and 4.2).

Throughout phenological development, TPN and GN increased over sampling time, but the rate of increase varied among locations (Figure 4.1; Appendix 4.2 and 4.3). The delay in remobilization of N until the milk stage at Goodale and Kernen, could be the result of cultivars heading and and ripening later than at Nasser (Table 4.3). The rapid decrease of N content in all vegetative organs observed at Nasser was likely because of low N supply and earlier leaf senescence (Martin de Molino *et al*., 1995). Changes in plant tissue N content (Figure 4.1) and changes in the rates of N remobilization, post-anthesis NUP and grain N accumulation (Figure 4.2) were consistent throughout phenological development. However, while N remobilization was observed following anthesis, post anthesis NUP was more pronounced after the dough stage, suggesting that evaluation of N remobilization and post-athesis NUP would be best carried out using the data of TPN at anthesis versus GN and VegN at physiological maturity.

Senescence represents the final stage of vegetative development characterized by transition from nutrient assimilation to nutrient remobilization (Feller and Fischer, 1994). Leaves, and especially the flag leaf play important roles as N sinks during grain protein synthesis in many grass (Austin *et al*, 1977; Cox *et al.*, 1985a; 1986; Papakosta and Garianas, 1991; Millet *et al*., 1992; Barneix and Guitman, 1993; Wang *et al*., 2003; Tahir and Nakata, 2005; Kade *et al*., 2005; Uauy *et al*., 2006) because 75% of the N is present in the forms of stromal (especially ribulose-1,5-bisphosphate carboxylase/oxygenase [Rubisco]) and thylakoidal proteins (Peoples and Dalling, 1988; Hörtensteiner and Feller, 2002). However, in this study, the amount of stored N throughout phenological development and the amount of the remobilized N from the stem (culm and leaf sheaths) was greater than from the lower leaves and flag leaf (Figure 4.1) suggesting the importance of the stem as N sink compared to leaves.

During grain development, a large amount of N is remobilized from the vegetative organs to the developing grains for protein synthesis (Mae, 1997; Zhu *et al*., 2007; Dingkuhn, 1996). In addition to N uptake during the vegetative phase, direct uptake of nitrate and ammonia from the soil after flowering also contributes to elevated GPC (Blackman and Payne, 1987). In this study, N remobilization from all vegetative organs to the grains was apparent following anthesis and the rate was dependent on

environment (Figure 4.2). Differences in soil N levels at Kernen, Goodale and Nasser (Table 4.1) resulted in differences in TPN and GN at physiological maturity, being 49. 5, 47.2 and 36.6 mg plant^{-1}, and 34.0, 31.4, 27.5 mg plant^{-1}, respectively (Appendix 4.3). Barbotin *et al*. (2005) reported that environmental factors are the main sources for variation in N remobilization in wheat. In this study, however, differences in soil N levels did not result in differences in the total amount of the remobilized N at physiological maturity, which averaged over all locations was 26.5 mg plant^{-1} (Table 4.5; Figure 4.2), such that differences in TPN and GN at physiological maturity among environments were attributed only to differences in post-anthesis NUP (Figure 4.2, Appendix 4.3). This result suggests a physiological interaction between N remobilization and post-anthesis NUP in influencing the grain N accumulation, and the interaction is genotype and environmental dependent. N remobilization was suppressed when post-anthesis NUP is high, and also the reverse (Cox *et al*., 1985a; 1985b).

As with previous reports from hexaploid wheat (Blackman and Payne, 1987; People and Dalling, 1988; Feller and Fischer, 1994; Clarke, 2005; Kichey *et al*., 2007), this study also found that the primary contribution to GPC in durum wheat was NR as reflected in the proportion of the remobilized N in the grains (GrainNR), being 73.6, 85.7 and 93.7% at Kernen, Goodale and Nasser, respectively (Table 4.5). Higher GrainNR was observed at Nasser where soil N was limiting, forcing the plants to make greater use of stored N. Nitrogen remobilization is most pronounced in situations of low N supply during the pre-flowering period (Barbottin *et al*., 2005). Under adverse conditions that limit photosynthesis and post-anthesis NUP, N remobilization from the vegetative organs functions as a buffer for grain N yield (Tahir and Nakata, 2005). On the other hand, sufficient soil N and available water at Kernen might have rendered N remobilization unnecessary. Indeed, higher levels of N fertilizer application before flowering have been reported to lead to decreased N remobilization (Cox *et al*., 1985a; 1985b). When soil N supply is adequate, high amino acid concentration in tissues represses N uptake machinery of the root resulting in low N uptake despite its availability (Barneix *et al*., 2007). This results in low N reduction, and amino acid export to the phloem is limited. The high N status of the plant maintains a high level of cytokinins, in such a way that leaf proteins and Rubisco are not degraded (Barneix *et al*.,

2007). As a consequence, the free amino acid export pool remains low, and the amino acid concentration and C/N ratio in the phloem do not decrease. At maturity, the GPC remains at its genetic potential level, while in the straw the N concentration is high (Barneix *et al*., 2007).

4.5.2. Effect of genotypes on agronomic traits, N remobilization and post-anthesis N uptake

The results presented here confirm that introgression of chromosome 6B from *T. durum* var. *dicoccoides* into Langdon resulted in increased GPC as shown in other studies (Joppa and Cantrell, 1990; Cantrell and Joppa, 1991, Joppa *et al*., 1997). In addition, Langdon(DIC-6B) showed a significantly greater amount of remobilized N than its isogenic counterpart Langdon (Table 4.10), confirming that the introgressed *GpcB-1* gene confers improved N remobilization to developing grain (Deckard *et al*., 1996; Kade *et al*., 2005). This gene was also reported to be associated with increased zinc, iron content, leaf senescence and enhanced N remobilization (Uauy *et al*., 2006a), likely the result of improved remobilization of the metals from senescing vegetative tissue. Among the check cultivars, Strongfield expressed high GPC and grain yield, which resulted in significantly higher grain protein yield. Similar to Langdon(DIC-6B) when compared with Langdon, Strongfield also had higher NR compared with DT695 and Commander. However, with the exception of Westbred 881, the proportion of NR to the total grain N of the check cultivars were similar (Table 4.10), suggesting that despite the major contribution of N remobilization, post-anthesis NUP was the determining factor for GPC difference among the check cultivars. No differences in NR were detected among high- and low-GPC DH selections, but at Kernen and Goodale, the high-GPC selections expressed siginificantly greater post-anthesis NUP.

Nitrogen harvest index, defined as the ratio between grain N yield and biomass N, can be used as a measure of N use efficency (Koutroubas *et al*., 2004). In wheat, cultivars with efficient N remobilization show high NHI, and consequently high GPC (Kichey *et al*., 2007). In this study, the high GPC check cultivars also expressed high NHI, and semi-dwarf cultivars show low NHI, but no difference in NHI ($P>0.05$) was observed between the high- and low-GPC DH selections (Table 4.10). This suggests

that, at least in the DH selections used here, that grain N yield is accumulated not only from N remobilization but also post-anthesis NUP.

In wheat, a considerable proportion of assimilated N used immediately in developing grain (post-anthesis NUP) has been reported elsewhere (Dupont and Altenbach, 2003; Kichey *et al*., 2007; Muurinen *et al*., 2007). However, genetic variation for post-anthesis NUP is smaller than variation due to growing conditions (Muurinen *et al*., 2007). In barley, post-anthesis NUP contributed 44 and 22% of total ear N in low N-fed and optimal N-fed plants, respectively (Egle *et al.,* 2007). However, high-GPC barley cultivars did not accumulate more N after heading than low-GPC cultivars (Bulman and Smith, 1994). Strongfield showed higher post-anthesis NUP compared to other check culitvars (Table 4.10). Based on contrast analysis, post-anthesis NUP of low-GPC DH selections was similar to DT695, but the post-anthesis NUP of high-GPC DH selections was greater than Strongfield (Table 4.10; Appendix 4.4). Given these two DH selections represent extremes for GPC, this result might suggest a transgressive segregation of genes for post-anthesis NUP among the DH lines, and that only Strongfield contributes positive alleles for post-anthesis NUP in this DH population. This would need to be confirmed in the complete mapping population used for analysis of GPC (Chapter 3).

In the genetic study (Chapter 3), marker *barc108* was found to be associated with the GPC QTL *QGpc.usw-A3* on chromosome 7A. When the DH selections used in this physiological study were analyzed for *barc108* (after the fact), it was observed that all the high-GPC DH selections carried the Strongfield allele at *barc108*, while the majority of low-GPC DH selections (except DAH-46* and DAQ-02*), had the *barc108* allele similar to DT695. This result coincides well with the previous finding that Strongfield contributed the positive allele for GPC QTL *QGpc.usw-A3* on 7A (Chapter 3). The post-anthesis NUP of DH selections carrying Strongfield allele at *barc108* was also significantly higher than DH selections carrying DT695 allele at *barc108* (Table 4.10). This may suggest the association of this QTL with post-anthesis NUP as all of the high GPC lines expressed greater NUP. However, more lines would have to be assessed in a detailed QTL study to confirm this. Nitrate reductase (NAR) and nitrite reductase (NIR) activities are highly correlated with post-anthesis NUP and GPC

(Kichey *et al*., 2007; Miflin and Habash, 2002; Good *et al*., 2004; Boisson *et al*., 2005). Two NADH-NAR genes have been reported in wheat localized on linkage groups 6 and 7 (Kilian *et al*., 1992; Habash *et al.*, 2007). However, the GPC QTL *QGpc.usw-A3* on 7A does not co-localize with the 7AS QTL reported for NAR (Habash *et al*., 2007; Chapter 3). A multi-location physiological study using chromosome 7A-deletion lines, combined with a green house study using ^{15}N, would be needed to confirm the association between chromosome 7A and post-anthesis NUP.

Elevated GPC is positively correlated with TPN at anthesis in rice (Ntanos and Koutroubas, 2002), and with free amino acid concentrations in the flag leaf during grain filling in wheat (Millet *et al*., 1992; Barneix and Guitman, 1993). In this study, Langdon(DIC-6B) had higher NR and more TPN-An compared with its isogenic counterpart Langdon (Table 4.8). Similarly, Strongfield had numerically higher TPN-An and less VegN-PM than most check cultivars at all locations, but this difference was not significant when averaged over locations (Table 4.8). In contrast, the two DH groups had similar TPN-An and NR, but the high-GPC DH selections had more remaining N in their vegetative organs at physiological maturity (VegN-PM) (Table 4.8), giving further evidence that high GPC in these DH selections was due to more capacity to absorb soil N during grain development. However, not all N was translocated to the grain, and some of the N remained in vegetative organs until physiological maturity (Table 4.8). Kichey *et al*. (2007) reported that about 9% of N absorbed post-flowering is not translocated to the grain and that this fraction of N that was variable among the five cultivars they evaluated. Interestingly, the low-GPC check cultivars tended to have more VegN-PM than the high-GPC checks, while the reverse occurred among the DH groups. This could suggest better post-anthesis NUP of the high-GPC DH selections was not accompanied by better N remobilization so that some of the absorbed N remained in the leaves until physiological maturity. This argument is supported by the fact that despite the difference in GPC, the NHI of the two DH groups was also similar (Table 4.10). The use of more DH lines that represent segregation in N remobilization may help confirm this hypothesis.

The *Rht-B1b* and *Rht-D1b* gibberellin insensitive dwarfing genes are widely used to reduce plant height and increase grain yield in wheat breeding programs (Botwright *et*

al., 2005). The cultivar Commander carries the *Rht-B1b* gene and epxressed significantly lower GPC than Strongfield (Table 4.7). Lines carrying this gene are characterized by concomitant reductions in the sub-crown internode and coleoptile length and leaf area of seedlings (Allan *et al*., 1961; Allan, 1989; Botwright *et al*., 2001), and express significantly reduced GPC (McClung *et al*., 1986; Pinthus and Gale, 1990). Averaged over three locations throughout phenological development, Commander and Westbred 881 had numerically lower N content in the stem compared to other check cultivars (Table 4.9). Westbred 881, which also carries *Rht-B1b*, had lower TPN-An but instead of having GPC similar to Commander, it had GPC similar to DT695 (Table 4.7). Westbred 881 headed and matured earlier than other check cultivars so that its high GPC was likely because of shorter grain filling period. Increased GPC under shortened duration of the grain filling period can be attributed mainly to reduced accumulation of starch (Jenner *et al*., 1991). However, only two semi-dwarf cultivars were included in this study, more semi-dwarf cultivars are required to further justify this hypothesis.

4.6. Conclusion

The present study demonstrated that genetic variation for elevated GPC is available in Canadian durum wheat germplasm. Strongfield has comparably better advantage over other Canadian durum cultivars, as well as over Langdon(DIC-6B), in GPC and grain yield resulting in its higher grain protein yield.

The present study confirmed that N remobilization was the primary sources of grain N in durum wheat, and stem was the primary source for N remobilization. However, differences in soil N levels result only in differences in post-anthesis NUP, but not in N remobilization. N remobilization was suppressed when post-anthesis NUP was high, and also the reverse.

The present study confirmed that that introgression of chromosome 6B from *T. durum* var. *durum* resulted in increased GPC, and this increase was due to higher N remobilization. Similarly, Strongfield has better N remobilization than DT695. However, differences in GPC between Strongfield and DT695 and the semi-dwarf cultivars were due to post-anthesis NUP. Strongfield and the high-GPC DH selections

from the cross DT695 x Strongfield demonstrated gretaer post-anthesis NUP than DT695 and the low-GPC DH selections. All six high-GPC DH selections carried the Strongfield allele at *QGpc.usw-A3,* and could suggest that this QTL is associated with post-anthesis NUP, but this will need to be confirmed in future studies. The semi-dwarf cultivars Commander and Westbred 881 had low GPC, and because they did not accumulate N post-anthesis, the majority of grain protein was derived only from N remobilization.

Chapter 5

GENERAL DISCUSSION

5.1. Research findings

A major objective of this thesis was to identify QTL for GPC to understand the genetic basis of elevated GPC and to identify DNA-based markers to enhance selection efficiency in durum wheat breeding programs (Chapter 3). Through genetic analysis, two stable QTL for GPC both derived from Strongfield on chromosome 2B and 7A were identified. Individually, the GPC QTL *QGpc.usw-B3* on 2B and *QGpc.usw-A3* on 7A produced an average effect on GPC of +0.23 and +0.30%, respectively. Another GPC QTL contributed by DT695 was *QGpc.usw-A2* on chromosome 2A having an average effect on GPC of +0.27%. All these QTL showed no negative pleiotropic effect for GPC and seed weight, but correlation with reduced yield was observed at SC 2002 and RG 2003 for *QGpc.usw-A2*, at ST 2005 for *QGpc.usw-B3*, and at RG 2003 for *QGpc.usw-A3* that may limit the use of these QTL as targets for selection.

The GPC QTL *QGpc.usw-B3* on 2B and *QGpc.usw-A3* on 7A identified in this study appear to be orthologous to those reported in separate bread wheat populations (Prasad *et al.*, 1999; Turner *et al.*, 2004). The *wmc41* associated with *QGpc.usw-B3* was previously validated as selection tool for high GPC QTL in bread wheat populations (Harjit-Singh *et al.*, 2001). However, this marker was monomorphic in the validation population in this study, suggesting this QTL was fixed. However, *QGpc.usw-A3* was validated in a DH population derived from the cross Strongfield x C9370-DJ**3. The *barc108*-7A QTL association was reported earlier in bread wheat (Turner *et al.*, 2004). This study also confirmed the association between *gwm339* and 2A QTL previously reported for gluten strength in the population of Kyle2*/Biodur (Knox *et al*., 2004), and likely similar with that identified in the RIL population of *T. durum* var *turgidum* x *T. durum* var, *dicoccoides* (Blanco *et al*., 2006).

Several gaps currently exist on the DT695 x Strongfiled genetic map, and thus additional QTL could be segregating in this population. Construction of a more saturated genetic map that covers all chromosomes, as well as identification of GPC

QTL in other durum wheat mapping populations may help to identify other useful GPC QTL. Identification of more QTL with major effects on GPC will provide an opportunity for QTL pyramiding program for elevation of GPC.

Strongfield is a Canadian durum wheat released in 2002 (Clarke *et al*., 2005), and has consistently displayed high GPC and high yield, hence it has been used extensively in Canadian durum wheat crossing programs. In both genetic (Charpter 3) and physiological (Chapter 4) studies, Strongfield consistently showed significantly higher GPC, as well as higher grain yield, resulted in higher grain protein yield than DT695 and the semi-dwarf cultivars Commander and Westbred 881 (Table 4.7). As expected, the Langdon(DIC-6B) consistently showed higher GPC than Langdon (Table 4.7) confirming the GPC QTL *Gpc-B1* on 6B chromosome of *T. durum* var *dicoccoides* has a positive effect on elevation of GPC through better N remobilization.

As with previous reports on the physiology of GPC (Austin *et al.*, 1977; Cox *et al.*, 1985a, 1986; Papakosta and Garianas, 1991), our result demonstrated that regardless of the genotypes and growing conditions, N remobilization was the primary contributor for grain N accumulation, accounted for an average of 84.3% of the GPC (Table 4.4). However, this study revealed that despite Strongfield is better than DT695 and the semi-dwarf cultivars Commander and Westbred 881 in N remobilization and post-anthesis NUP, the elevated GPC of the Cultivar Strongfield was only due to better pos-anthesis NUP.

In the QTL study (Chapter 3), a microsatellite marker *barc108* was associated with GPC QTL *QGpc.usw-A3* on chromosome 7A. Further analysis with the DH groups used in the physiological study showed that all the high-GPC DH selections have *barc108* allele similar to Strongfield, while the low-GPC DH selections, except DAH-46* and DAQ-02*, have *barc108* allele similar to DT695. This result corresponds with the previous finding that Strongfield contributed the positive allele for GPC QTL *QGpc.usw-A3* on 7A (Chapter 3) and post-anthesis NUP (Chapter 4). However, the GPC QTL *QGpc.usw-A3* on 7A does not co-localize with the 7AS QTL reported for NAR (Habash *et al*., 2007; Chapter 3), so that the positive allele for post-anthesis NUP in the cultivar Strongfield is possibly associated with other NAR genes not localized on

chromosome 7A, or with NIR genes as no NIR genes or QTL have been reported on wheat (Boisson *et al*., 2005).

5.2. Future research

Despite the significance of major QTL identified in this study, it is recognized that GPC QTL with small effects might have been overlooked as the genetic map used for the present QTL analysis did not cover the whole genome. It is also recognized that QTL with major effect for GPC may also be conserved in durum cultivars other than Strongfield and DT695. Therefore, identification of GPC QTL using a more saturated genetic map, as well as identification of GPC QTL in other durum wheat populations is important if breeding for elevated GPC is to be conducted via gene pyramiding.

The *barc108* was validated to be consistently associated with the *QGpc.usw-A3* QTL on 7A in at least two populations related to Strongfield. There is no guarantee that DNA markers identified in one population will be useful in different population. Therefore, validation of *barc108* should involved independent and different genetic background, as well as more diverse environments. Production of near isogenic lines (NILs) for the *QGpc.usw-A3* QTL in multiple genetic backgrounds should be examined to confirm the expression of this QTL in those backgrounds. Confirmation would further warrant efforts to pursue finer mapping and positional cloning using the NILs to elucidate the gene(s) associated with elevated GPC at this QTL. In addition, these NILs could be used to better understand the physiological mechanisms associated with elevated GPC as the result of this QTL.

This study demonstrated that depending on cultivar, elevated GPC in durum wheat is brought about by efficient N remobilization, as well as by post-anthesis NUP. N remobilization and post-anthesis NUP are also controlled by complex genetic systems. Therefore, identification of QTL for N remobilization and post-anthesis N uptake would be another approach for breeding for elevated GPC. Confirmation of association between *Rht-B1* gene and low GPC and stem as the predominant N sink that involve more semi-dwarf and tall cultivars would also be another important study.

5.3. Conclusions and novel scientific contributions

The present study reported two stable QTL for GPC *QGpc.usw-B3* on chromosome 2B and *QGpc.usw-A3* on chromosome 7A derived from the cultivar Strongfield, a Canadian durum wheat cultivars that has been widely used in durum crossing programs. The fact that the low GPC parent DT695 also contributed positive alleles for GPC, *QGpc.usw-A2* on chromosome 2A, suggests that alleles for elevated GPC may present in any Canadian durum wheat cultivars, and highlights the possibility of exploring novel QTL for elevated GPC. Eventually, identification of novel GPC QTL from other cultivars and breeding lines will help durum wheat breeders efficiently select the appropriate genotypes for their crossing programs aiming at elevation of GPC through QTL pyramiding.

The present study confirmed N remobilization to be the primary source for grain N, and elevated GPC in Langdon(DIC-6B) was due to the introgression of *Gpc-B1* gene (chromosome DIC-6B) that confers improved N remobilization. However, N remobilization is not the only determining factor for elevated GPC in durum wheat. Compared with DT695 and the low-GPC DH selections, Strongfield and the high-GPC DH selections had higher post-anthesis NUP, supporting the hypothesis that elevated GPC in Strongfield is derived from greater post-anthesis NUP.

LITERATURE CITED

AACC (2000) AACC Method 44-15A. Moisture content. AACC, 10th edition. American Association of Cereal Chemists. St.Paul, MN. P: 1-4.

AACC (2000) AACC Method 46-30. Crude protein - Combustion method. AACC, 10th edition. American Association of Cereal Chemists. St.Paul, MN. P: 1-2.

AAFC (2005) Durum wheat: 2005-2006 situation and outlook. Bi-weekly Bulletin. Agriculture Agri-Food Canada. December 16, 2005. Vol. 18. No. 20.

Allan RE (1989) Agronomic comparisons between *Rht1* and *Rht2* semidwarf genes in winter wheat. Crop Science 29: 1103-1108.

Allan RE, Vogel O, Burleigh J, Peterson C (1961) Inheritance of coleoptile length and its association with culm length in four winter wheat crosses. Crop Science 1: 328-332.

Amarasinghe BHRR, Debruxella GL, Braddon M, Onyeocha I, Forde BG, Udvardi MK (1998) Regulation of GMNRT2 expression and nitrate transport activity in soybean (*Glycine max*). Planta 206: 44-52.

Arduini I, Masoni A, Ercoli L, Mariotti M (2006) Grain yield, and dry matter and nitrogen accumulation and remobilization in durum wheat as affected by variety and seeding rate. European Journal of Agronomy 25: 309-318.

Austin RB, Ford MA, Edrich JA, Blackwell R D (1977) The nitrogen economy of winter wheat. Journal of Agricultural Science 88: 159-167.

Autran JG, Abecassis J, Feillet P (1986) Statistical evaluation of different technological and biochemical tests for quality assessment in durum wheats. Cereal Chemistry 63: 390-394.

Avila C, Marquez AJ, Pajuedo P, Cannel ME, Wallsgrove RM, Forde BG (1993) Cloning and sequence analysis of a cDNA for barley ferredoxin-dependent glutamate synthase and molecular analysis of photorespiratory mutants deficient in the enzyme. Planta 189: 475-483.

Avivi L (1978) High protein content in wild tetraploid *Triticum dicoccoides* Korn. In: S. Ramanujam (ed.), International Wheat Genetic Symposium, 5th ed. Indian Soc. Genet. Plant Breed., New Delhi, India. P: 372-380.

Barbottin A, Lecomte C, Bouchard C, Jeuffroy MH (2005) Nitrogen Remobilization during Grain Filling in Wheat: Genotypic and Environmental Effect. Crop Science 45: 1141-1150.

Baresel JP, Zimmermann G, Reents HJ (2008) Effects of genotype and environment on N uptake and N partition in organically grown winter wheat (*Triticum aestivum* L.) in Germany. Euphytica 163: 347-354.

Barneix AJ (2007) Physiology and biochemistry of source-regulated protein accumulation in the wheat grain. Journal of Plant Physiology 164: 581-590.

Barneix AJ, Guitman MR (1993) Leaf regulation of the nitrogen concentration in the grain of wheat plants. Journal of Experimental Botany 44: 1607-1612.

Barker AV, Bryson GM (2007) Nitrogen. In: Barker AV, Pilbeam DJ (eds.) Handbook of Plant Nutrition. Taylor & Francis. Boca Ratoon, FL. P: 21-50.

Beninati NF, Busch RH (1992) Grain protein inheritance and nitrogen uptake and redistribution in a spring wheat cross. Crop Science 32: 1471-1475.

Bietz JA, Wall JS (1980) Identity of high molecular weight gliadin and ethanol soluble glutenin subunits of wheat: relation to gluten structure. Cereal chemistry 57: 415-421.

Bezant JH, Laurie DA, Pratchett N, Kearsey MJ (1997) Mapping of QTL controlling NIR predicted hot water extract and grain nitrogen content in a spring barley cross using marker-regression. Plant Breeding 116: 141-145.

Bhatt GM, Derera FN (1975) Genotype x Environment interactions for heritabilities of, and correlations among quality traits in wheat. Euphytica 24: 597-604.

Bhave M, Morris CF (2007) Molecular genetics of puroindolines and related genes: allelic diversity in wheat and other grasses. Plant Molecular Biology 66: 205-219.

Blackman JA, Payne PI (1987) Grain quality. In: Heyne EG (ed.) Wheat Breeding. Its Scientific Basis. Chapman and Hall Ltd., London. P: 455-485.

Blanco A, Simeone R, Gadaleta A (2006) Detection of QTLs for grain protein content in durum wheat. Theoretical and Applied Genetics 112: 1195-1204.

Blanco A, Pasqualone A, Troccoli A, Di Fonzo N and Simeone R (2002) Detection of grain protein content QTLs across environments in tetraploid wheats. Plant Molecular Biology 48: 615-623.

Blanco A, Bellomo MP, Lotti C, Pasqualone A (1998) Mapping of quantitative trait loci for grain quality using recombinant inbred lines of durum wheat. EWAC Newsletter P: 106-110.

Blanco A, Giovanni de C, Laddomada B, Sciancalepsre A, Simeone R, Devos KM, Gale MD (1996) Quantitative trait loci influencing grain protein content in tetraploid wheat. Plant Breeding 115: 310-316.

Boisson M, Mondon K, Torney V, Nicot N, Laine AL, Bahrman N, Gouy A, Daniel-Vedele F, Hirel B, Sourdille P, Dardevet M, Ravel C, Le Gouis J (2005) Partial sequences of nitrogen metabolism genes in hexaploid wheat. Theoretical and Applied Genetics 110: 932-940.

Botwright TL, Rebetzke GJ, Condon AG, Richards RA (2005) Influence of the Gibberellin-sensitive *Rht8* Dwarfing Gene on Leaf Epidermal Cell Dimensions and Early Vigour in Wheat (*Triticum aestivum* L.). Annals of Botany 95: 631-639.

Botwright TL, Rebetzke G, Condon T, Richards RA (2001) The effect of rht genotype and temperature on coleoptile growth and dry matter partitioning in young wheat seedlings. Australian Journal of Plant Physiology 28: 417-423.

Branlard G, Autran JC, Monneveux P (1989) High molecular weight glutenin subunit in durum wheat (*T. durum*). Theoretical and Applied Genetics 78: 353-358.

Brites C, Carrillo JM (2001) Influence of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits controlled by *Glu-1* and *Glu-3* loci on durum wheat quality. Cereal Chemistry 78: 59-63.

Brouquisse R, Masclaux C, Feller U, Raymond P (2001) Protein hydrolysis and nitrogen remobilization in plant life and senescence. In: Lea PJ and Morot-Gaudry JF (eds.) Plant Nitrogen. Berlin: Springer. P: 275-293.

Brown JWS, Flavell RB (1981) Fractionation of wheat gliadin and glutenin subunits by two-dimensional electrophoresis and the role of group 6 and group 2 chromosome in gliadin synthesis. Theoretical and Applied Genetics 59: 349-359.

Bulman P, Smith DL (1994) Grain protein response of malting barley to high rates and post-anthesis application of fertilizer nitrogen. Agronomy Journal 85: 1109-1113.

Buonocore V, De Biasi MG, Giardina P, Poerio E, Silano V (1985) Purification and properties of an alpha-amylase tetrametric inhibitor from wheat kernel. Biochimica et Biophysica Acta (BBA) 831: 40-48.

Bush DR (1999) Amino acid transport. In: Singh BK (ed.) Plant Amino Acids: biochemistry and biotechnology. Marcel Dekker Inc., New York. P: 305-318.

Bushuk W, Zillmann RR (1978) Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. Canadian Journal of Plant Science 58: 505-515.

Callis J (1995) Regulation of protein degradation. The Plant Cell 7: 845-857.

Campbell CA, Zentner RP, Janzen HH, Bowren KE (1990) Crop rotation studies on the Canadian prairie. Agriculture Canada Pub. 1481/E. Ottawa, Ontario. P: 133.

Campbell CA, Davidson HR, Winkelman HR (1981) Effect of nitrogen, temperature, growth stage and duration of moisture stress on yield components and protein content of Manitou spring wheat. Canadian Journal of Plant Science 61: 549-563.

Cantrell RG, Joppa LR (1991) Genetic analysis of quantitative traits in wild emmer (*Triticum turgidum* L. var *dicoccoides*). Crop Science 31: 645-649.

Caputo C, Barneix AJ (1997) Amino acids export to the phloem in relation to N supply in wheat (*Triticum aestivum* L.) plants. Physiologia Plantarum 101: 853-860.

Carillo P, Mastrolonardo G, Nacca F, Fuggi A (2005) Nitrate reductase in durum wheat seedlings as affected by nitrate nutrition and salinity. Functional Plant Biology 32: 209-219

Charmet G, Groos C (2002) QTLs in bread making quality: a review. In: Mohan Jain S,

Brar DS, Ahloowalia BS (eds.) Molecular Techniques in crop improvement. Kluwer Academic Publisher. Dordrecht. The Netherlands. P: 601-616.

Chee PW, Elias EM, Anderson JA, Kianian SF (2001) Evaluation of high grain protein content QTL from *Triticum turgidum* L. var. *dicoccoides* in an adapted durum wheat background. Crop Science 41: 295-301.

Cheng C, Dewdney J, Nam H, den Boer BG, Goodman HM (1988) A new locus (*NIA 1*) in *Arabidopsis thaliana* encoding nitrate reductase. EMBO Journal 7: 3309-3314.

Ciaffi M, Dominici L, Lafiandra D, Porceddu E (2008) Seed storage proteins of wild wheat progenitors and their relationships with technological properties. Hereditas 116: 315-322.

Ciaffi M, Lee YK, Tamas L, Gupta R, Skerritt J, Appels R (1999) The low-molecular-weight glutenin subunit proteins of primitive wheats. III. The genes from D-genome species. Theoretical and Applied Genetics 98:135-148.

Ciaffi M, Benedettelli S, Giorgi B, Porceddu E, Lafiandra D (1991) Seed storage proteins of *Triticum durum* spp. *dicoccoides* and their effect on technological quality in durum wheat. Plant Breeding 107: 309-319.

Clarke FR, Clarke JM, McCaig TN, Knox RE, DePauw RM (2006) Inheritance of yellow pigment in concentration in four durum wheat crosses. Canadian Journal of Plant Science 86: 133-141.

Clarke JM, Knox RE, DePauw RM, Clarke FR, Fernandez MR, McCaig TN, Singh AK (2009). Brigade durum wheat. Canadian Journal of Plant Science 89: 505-509.

Clarke JM, McCaig TN, DePauw RM, Knox RE, Ames NP, Clarke FR, Fernandez MR, Marchylo BA, Dexter JE (2006) Registration of 'Commander' Durum Wheat. Crop Science 46: 2701.

Clarke JM (2005) Durum wheat improvement in Canada. In: Royo C, Nachit MM, Di Fonzo N, Araus JL, Pfeiffer WH, Slafer GA (eds.) Durum wheat breeding. Current approaches and future strategies. Vol.2. P: 921-938.

Clarke JM, McCaig TN, DePauw RM, Knox RE, Clarke FR, Fernandez MR, Ames NP (2005) Strongfield durum wheat. Canadian Journal of Plant Science 85: 651-654.

Clarke JM, Mcleod JG, DePauw RM, Marchylo BA, McCaig TN, Knox RE, Fernandez MR and Ames N (2001) Registration of `AC Navigator' Durum Wheat. Crop Science 41: 270-271.

Collaku A, Harrison SA (2005) Heritability of waterlogging tolerance in wheat. Crop Science 45: 722-727.

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142: 169-196.

Colmer TD, Flowersand TJ, Munns R (2006) Use of wild relatives to improve salt tolerance in wheat. Journal of Experimental Botany 57: 1059-1078.

Coschigano KT, Melo-Oliveira R, Lim J, Coruzzi GM (1998) *Arabidopsis* gls mutants and distinct Fd-GOGAT genes. Implications for photorespiration and primary nitrogen assimilation. Plant Cell 10:741–752

Cox MC, Qualseet CO, Rains DW (1985a) Genetic variation for nitrogen assimilation and translocation in wheat. I. Dry matter and nitrogen accumulation. Crop Science 25: 430-435.

Cox MC, Qualseet CO, Rains DW (1985b) Genetic variation for nitrogen assimilation and translocation in wheat. II. Nitrogen assimilation in relation to grain yield and protein. Crop Science 25: 435-440.

Cox MC, Qualseet CO, Rains DW (1986) Genetic variation for nitrogen assimilation and translocation in wheat. III. Nitrogen translocation in relations to grain yield and protein. Crop Science 26: 737-740.

Dachkevitch T, Redaelli R, Biancardi AM, Metakovsky EV, Pogna NE (1993) Genetics of the gliadins coded by the group 1 chromosomes in high-quality bread wheat cultivar 'Neepawa'. Theoretical and Applied Genetics 86: 389-399.

Dalling MJ (1985) The physiological basis of nitrogen redistribution during grain filling in cereals. In: Harper JE, Schrader LE and Howell RW (eds.) Exploitation of Physiological and Genetic Variability to Enhance Crop Productivity. MD, USA. American Society of Plant Physiologists. P: 55-69.

Damidaux R, Autran JC, Grignac P, Feillet P (1978) Evidence of relationships useful for breeding between the electrophoretic pattern of gliadins and the viscoelastic properties of the gluten in *Triticum durum* Desf. C.R. Academy of Sciences, Paris. 287: 701-704.

Damodaran S (1996) Amino acids, peptides, and proteins. In: Fennema OR (ed.) Food Chemistry. CRC Press. P: 321-430.

Deckard EL, Joppa LR, Hammond JJ, Hareland GA (1996) Grain protein determinants for Langdon durum-*dicoccoides* chromosome substitution lines. Crop Science 36: 1513-1516.

D'Egidio MG, Mariani BM, Nardi S (1990) Chemical and technological variables and their relationships: a predictive equation for pasta cooking quality. Cereal Chemistry 67: 275-281.

Deng M-D, Moureaux T, Leydecker M-T, Caboche M (1990) Nitrate-reductase expression is under the control of a circadian rhythm and is light inducible in *Nicotiana tabacum* leaves. Planta 180: 257-261.

DePauw RM, Knox RE, Clarke FR, Wang H, Fernandez MR, Clarke JM, McCaig TN (2007) Shifting undesirable correlations. Euphytica 157: 409-415.

DePauw RM, Townley-Smith TF, Humphreys G, Knox RE, Clarke FR, Clarke JM (2005) Lillian hard red spring wheat. Canadian Journal of Plant Science 85: 397-401

DePauw RM, Clarke JM, McCaig TN, Townley-Smith TF (1998). Opportunities for the improvement of western Canadian wheat protein concentration, grain yield and quality through plant breeding. *In*: Fowler, DB, Geddes WE, John AM, Preston KR (eds). Wheat protein production and marketing. Proc. of the Wheat Protein Symposium. March 9 and 10, 1998. Saskatoon, SK, Canada. P: 75-93.

De Stefanis E, Sgrulletta D, Cobellini M, Di-Fonzo N, Ronga G *(*1998) Accumulation of acetic acid and insoluble protein in grain of *T. durum* cultivars grown under heat stress conditions. Journal of Genetics and Breeding 52: 343-352.

De Stefanis E, Sgrulletta (1990) Effect of high-temperature drying on technological properties of pasta. Journal of Cereal Science 12: 97-104.

Dexter JE, Dronzek BL (1975a) Amino acid composition of maturing endosperm from hexaploid triticale and its spring rye and durum wheat parents. Canadian Journal of Plant Science 55: 537-546.

Dexter JE, Dronzek BL (1975b) Protein synthesis in triticale and its durum wheat and rye parents. Cereal Chemistry 52: 577-586.

Dexter JE, Edwards NM (1999) The implications of frequently encountered grading factors on the processing quality of durum wheat. Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Manitoba, Canada R3C 3G8. Contribution No M231. Association of Operative Millers - Bulletin. http://www.grainscanada.gc.ca/Pubs/confpaper/Dexter/Grading/dgradinge. htm#abstract. Website was accessed on May 11, 2008.

Dexter JE, Matsuo RR (1980) Relationship between durum wheat protein properties and pasta dough rheology and spaghetti cooking quality. Journal of Agriculture and Food Chemistry 28: 899-902.

Dexter JE, Matsuo R.R (1977) Influence of protein content on some durum wheat quality parameters. Can J Plant Sci 57: 717-727.

Dholakia BB, Ammiraju JSS, Santra DK, Singh H, Katti MV, Lagu MD, Tamhankar SA, Rao VS, Gupta VS, Dhaliwal HS, Ranjekar PK (2001) Molecular marker analysis of protein content using PCR-based markers in wheat. Biochemical Genetics 39: 325-338.

Dimmock JPRE, Gooding MJ (2002) The influence of foliar diseases, and their control by fungicides, on the protein concentration of wheat grain: a review. Journal of Agriclutural Science 138: 349-366.

Dingkuhn M (1996) Modelling concepts for the phenotypic plasticity of dry matter and nitrogen partitioning in rice. Agricultural Systems 52: 383-397.

Distefano S, Palma JM, McCarthy I, del Rio LA (1999) Proteolytic cleavage of plant proteins by peroxisomal endoproteases from senescent pea leaves. Planta 209: 308-313.

Distelfeld A, Korol A, Dubcovsky J, Uauy C, Blake T, Fahima T (2008) Colinearity between the barley grain protein content (GPC) QTL on chromosome arm 6HS and the wheat *Gpc-B1* region. Molecular Breeding 22: 25-38.

Distelfeld A, Uauy C, Fahima T, Dubcovsky J (2006) Physical map of the wheat high-grain protein content gene *Gpc-B1* and development of a high-throughput molecular marker. New Phytologist 169: 753-763.

Distelfeld A, Uauy C, Olmos S, Schlatter AR, Dubcovsky J, Fahima T (2004) Microcolinearity between a 2-cM region encompassing the grain protein content locus *Gpc-6B1* on wheat chromosome 6B and a 350-kb region on rice chromosome 2. Funct Integr Genomics 4: 59-66.

D'Ovidio R (1993) Single-seed PCR of LMW glutenin genes to distinguish between durum wheat cultivars with good and poor technological properties. Plant Molecular Biology. 22: 1173-1176.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Du Cros DL, Wrigley CW, Hare RA (1987) Prediction of durum wheat quality from gliadin-protein composition. Australian Journal of Agricultural Research. 33: 429-442.

Duke SH, Duke SO (1984) Light control of extractable nitrate reductase activity in higher plants. Physioligia Plantarum 62: 485-493.

Dupont FM, Altenbach SB (2003) Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. Journal of Cereal Science 38: 133-146.

Egle K, Beschow H, Merbach W (2007) Assessing post-anthesis nitrogen uptake, distribution and utilization in grain protein synthesis in barley (*Hordeum vulgare* L.) using 15N fertilizer and 15N proteinogenic and non-proteinogenic amino acids. Annal of Applied Biology 152: 209-221.

Elias EM, Manthey FA (2005) End products: present and future uses. In: Mohan Jain S, Brar DS, Ahloowalia BS (Eds.) Molecular Techniques in Crop Improvement. Kluwer Academic Publishers. Dordrecht, The Netherlands. P: 63-86.

Elouafi I, Nachit MM (2004) Genetic linkage map of the durum x *Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits. Theoretical and Applied Genetics 108: 401-413.

Elouafi I, Nachit MM, Martin LM (2001) Identification of a microsatellite on chromosome 7B showing a strong linkage with yellow pigment in durum wheat (*Triticum turgidum* L. var. *durum*). Hereditas 135: 255-261.

Ellis MH, Rebetzke G, Chandler P, Bonnett D, Spielmeyer W, Richards R (2004) The effect of different height reducing genes on early growth of wheat. Functional Plant Biology 31: 583-589.

Ellis MH, Spielmeyer W, Gale KR, Rebetzke GJ, Richards RA (2002) 'Perfect' markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. Theoretical and Applied Genetics 105: 1038-1042.

Emebiri LC, Moody DB, Panozzo JF, Chalmers KJ, Kretschmer JM, Ablett GA (2003) Identification of QTLs associated with variation in grain protein concentration in two-row barley. Australian Journal of Agricultural Research 54: 1211-1221.

Fatta N, Caputo C, Barneix AJ (2000) The absence of the short arm of chromosome 7B produces inhibition of N mobilization and decrease grain protein concentration in wheat (*Triticum aestivum* L.) cv. Chinese Spring. Agronomie 10: 423-430.

Feillet P (1988) Protein and enzyme composition of durum wheat. In: Fabriani G and Lintas C (eds.) Durum Wheat: Chemistry and Technology. AACC. St Paul, MN. P: 93-119.

Feillet P, Dexter JE (1996) Quality requirements of durum wheat for semolina milling and pasta production. In: Kruger JE, Matsuo RR, Dick JW (eds.) Pasta and Noodle Technology. AACC. St. Paul, MN. P: 95-131.

Feller U, Fischer A (1994) Nitrogen metabolism in senescing leaves. Critical Reviews in Plant Sciences 13: 241-273.

Fernandez-Figares I, Marinetto J, Royo C, Ramos JM, Garcia del Moral LF (2000) Amino-acid composition and protein and carbohydrate accumulation in the grain of triticale grown under terminal water stress simulated by a senescing agent. Journal of Cereal Science 32: 249-258.

Ficarelli A, Tassi F, Restivo FM (1999) Isolation and characterization of two cDNA clones encoding for glutamate dehydrogenase in *Nicotiana plumbaginifolia*. Plant Cell Physiology 40: 339-342.

Filleur S, Daniel-Vedele F (1999) Expression analysis of a high-affinity nitrate transporter isolated from *Arabidobsis thaliana* by differential display. Planta 207: 461-469.

Flintham JE, Boerner A, Worland AJ, Gale MD (1997) Optimizing wheat grain yield: effects of Rht (gibberellin-insensitive) dwarfing genes. Journal of Agricultural Science 128: 11-25.

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, Sela H, Fahima T, Dubcovsky J (2009) Kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323: 1357-1360.

Gale MD, Youssefian S (1985) Dwarfing genes in wheat. In: Russell GE (ed.). Progress in Plant Breeding. London. Butterworth. P: 1-35.

Galova Z., Smolkova H, Michalik I (1999) Formation of protein complex during grain wheat maturation. Rostlinna Vyroba 45: 183-188.

Galterio G, Grita L, Brunori A (1993) Pasta-Making Quality in *Triticum durum*. New Indices from the Ratio among Protein Components Separated by SDS-PAGE. Plant Breeding 110: 290-296.

Ganal MW, Röder MS (2007) Microsatellite and SNP markers in wheat breeding. Varshney RK, Tuberosa R (eds.) Genomics-Assisted Crop Improvement, Vol 2: Genomics Applications in Crops. Springer Netherlands. P: 1-24.

Gao LF, Jing RL., Huo NX, Li Y, Li XP, Zhou RH, Chang XP, Tang JF, Mang ZY, Jia JZ. (2004) One hundred and one new microsatellite loci derived from ESTs (EST-SSRs) in bread wheat. Theoretical and Applied Genetics 108: 1392-1400.

Garcia-Olmedo F, Carmona MJ, Lopez-Fando JJ, Fernandez JA, Castagnaro A, Molina A, Hernandez-Lucas C, Carbonero P (2002) Characterization and analysis of thionin genes. In: Boller T, Molina A (eds.) Plant Gene Research. Genes Involved in Plant Defense. New York, Springer. P: 283-302.

Gautier MF, Cosson P, Guirao A, Alary R, Joudrier P (2000) Puroindoline genes are highly conserved in diploid ancestor wheats and related species but absent in tetraploid *Triticum* species. Plant Science 153: 81-91.

Gepstein S (2004) Leaf senescence: not just a wear and tear phenomenon. Genome Biology 5: 212.1-212.3.

Gianibelli MC, Larroque OR, MacRitchie, Wrigley CW (2001) Biochemical, genetic, and molecular characterization of wheat endosperm proteins. Online Review. Publication no. C-2001-0926-01O. AACC, Inc.

Giroux MJ, Talbert LE, Habernicht DK, Lanning SP, Hemphill A, Martin JM (2000) Association of puroindoline sequence type and grain hardness in hard red spring wheat. Crop Science 40: 370-374.

Gonzalez-Hernandez JL, Elias EM, Kianian SF (2004) Mapping genes for grain protein concentration and grain yield on chromosome 5B of *Triticum turgidum* (L.) var. *dicoccoides*. Euphytica 139: 217-225.

Good AG, Shrawat AK, Muench DG (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends in Plant Science 9: 597-605.

Gooding MJ, Davies WP (1997) Wheat production and utilization. Systems, quality and the environment. CAB International. Cambridge. UK.

Gowri G, Campbell WH (1989) cDNA clones for corn leaf NADH: nitrate reductase and chloroplast NAD(P)(+): glyceraldehyde-3-phosphate dehydrogenase. Plant Physiology 90: 792-798.

Grant CA, Flaten DN (1998) Fertilizing for protein content in wheat. *In* D.B. Fowler et al. (eds.) Wheat production and marketing, Proceedings of the Wheat Protein Symposium, Saskatoon, SK. March 9-10, 1998. Univ. of Saskatchewan Extension Press, Saskatoon, SK. P: 151-168.

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theoretical and Applied Genetics 106: 1032-1040.

Grzybowski RA, Donnelly BJ (1979) Cooking properties of spaghetti: factors affecting cooking quality. Journal of Agricultural and Food Chemistry 27: 380-384.

Gupta PK, Varshney RK, Prasad M (2002) Molecular markers: principles and methodology. In: Mohan Jain S, Brar DS, Ahloowalia BS (eds.) Molecular Techniques in Crop Improvement. Kluwer Academic Publisher. Dodrecht, The Netherlands. P: 9-54.

Gupta RB, Khan K, MacRitchie F (1993) Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. Journal of Cereal Science 18: 23-41.

Gupta RB, Batey IL, MacRitchie F (1992) Relationships between protein composition and functional properties of wheat flours. Cereal Chemistry 69: 125-131.

Gupta RB, Shepherd KW (1990a) Two-step one-dimensional SDSPAGE analysis of LMW subunits of glutenin. 1. Variation and genetic control of the subunits in hexaploid wheats. Theoretical and Applied Genetics 80: 65-74

Gupta RB, Shepherd KW (1990b) Two-step one-dimensional SDSPAGE analysis of LMW subunits of glutenin. 2. Genetic control of the subunits in species related to wheat. Theoretical and Applied Genetics 80: 183-187.

Gupta RB, Singh NK, Shepherd KW (1989) The cumulative effect of allelic variation in LMW and HMW glutenin subunits on dough properties in the progeny of two bread wheats. Theoretical and Applied Genetics 77: 57-64.

Habash DZ, Bernard S, Schondelmaier J, Weyen J, Quarrie SA (2007) The genetics of nitrogen use in hexaploid wheat: N utilization, development and yield. Theoretical and Applied Genetics 114: 403-419.

Harjit-Singh H, Prasad M, Varshney RK, Roy JK, Balyan HS, Dhalmal HS, Gupta PK (2001) STMS markers for grain protein content and their validation using near-isogenic lines in bread wheat. Plant Breeding 120: 273-278

Haynes RJ, Goh KM (1978) Ammonium and nitrate nutrition of plants. Biological Review 53: 465-510.

Hentschel V, Kranl K, Hollman J, Lindhauer MG, Bohm V, Bitsch R (2002) Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. Journal of Agricultural and Food Chemistry 50: 6663-6668.

Hessler TG, Thomson MJ, Benscher D, Nachit MM, Sorrells ME (2002) Association of a lipoxygenase locus, *Lpx-B1*, with variation in lipoxygenase activity in durum wheat seeds. Crop Science 42: 1695-1700

Hirel B, Bertin P, Quillere I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Cadiou S, Retailliau C, Falque M, Gallais A (2001) Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. Plant Physiology 125: 1258-1270.

Hoogendoorn J, Rickson JM, Gale MD (1990) Differences in leaf and stem anatomy related to plant height of tall and dwarf wheat (*Triticum aestivum* L.). Journal of Plant Physiology 136: 72-77.

Hörtensteiner S, Feller U (2002) Nitrogen metabolism and remobilization during senescence. Journal of Experimental Botany 53: 927-937.

Hu Zhong-Li, Li Ping, Zhou Ming-Quan, Zhang Zhi-Hong, Wang Ling-Xia, Zhu Li-Huang, Zhu Ying-Guo (2004) Mapping of quantitative trait loci (QTLs) for rice protein and fat content using doubled haploid lines. Euphytica 135: 47-54.

Humphreys DG, Procunier JD, Mauthe W, Howes NK, Brown PD, McKenzie RIH (1998) Marker-assited selection for high protein concentration in wheat. In: Fowler DB, Geddes WE, Johnston AM, Preston KR (eds.) Wheat protein production and marketing. Proc. of the Wheat Protein Symposium. March 9 and 10, 1998. Univ. Extension Press. Univ. of Saskatchewan, Saskatoon, Canada. P: 255-258.

Ireland RJ, Lea PJ (1999) The enzymes of glutamine, glutamate, asparagine, and aspartate metabolism. In: Singh BK (ed.) Plant amino acids. Biochemistry and biotechnology. Marcel Dekker Inc. New York. P: 49-109.

Jenner CF, Uglade TD, Aspinall D (1991) The physiology of starch and protein deposition in the endosperm of wheat. Australian Journal of Plant Physiology 18: 211-226.

Jochum JW, Busch RH, Fulcher GG, Hareland GA (2001) Recurrent selection for kernel weight in spring wheat. Crop Science 41: 999-1005.

Johansson E, Prieto-Linde ML, Jonsson JO (2001) Effects of wheat cultivar and nitrogen application on storage protein composition and breadmaking quality. Cereal Chemistry 78: 19-25.

Joppa LR, Cantrell RG (1990) Chromosomal location of genes for grain protein content of wild tetraploid wheat. Crop Science 30: 1059-1064.

Joppa LR, Du C, Hart GE, Hareland GA (1997) Mapping gene(s) for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines. Crop Science 37: 1586-1589.

Joppa LR, Walsh DE (1974) Quality characteristics of tall and semidwarf near-isogenic lines of durum wheat. Crop Science 14: 420-422.

Joppa LR, Khalil K, Williams ND (1983) Chromosomal location of genes for gliadin polypeptides in durum wheat *Triticum turgidum* L. Theoretical and Applied Genetics 64: 289-293.

Jukanti AK, Fisher AM (2008) A high-grain protein content locus on barley (*Hordeum vulgare*) chromosome 6 is associated with increased flag leaf proteolysis and nitrogen remobilization. Physiologia Plantarum 132: 426-439.

Kade M, Barneix AJ, Olmos S, Dubcovsky J (2005) Nitrogen uptake and remobilization in tetraploid 'Langdon' durum wheat and a recombinant substitution line with the high grain protein gene *Gpc-B1*. Plant Breeding 124: 343-349.

Kerber ER, Tipples KH (1969) Effects of the D genome on milling and baking properties of wheat. Canadian Journal of Plant Science 49: 255-263.

Keyes G, Sorrells ME, Setter TL (1990) Gibberellic acid regulates cell wall extensibility in wheat (*Triticum aestivum* L.). Plant Physiology 92: 242-245.

Keyes GJ, Paolillo DJ Jr, Sorrells ME (1989) The effects of dwarfing genes *Rht1* and *Rht2* on cellular dimensions and rate of leaf elongation in wheat. Annals of Botany 64: 683-690.

Khan IA, Procunier JD, Humphreys DG, Tranqulli G, Schlatter AR, Marcucci-Poltri S, Frohberg R, Dubcovsky J (2000) Development of PCR-based markers for a high grain protein content gene from *Triticum turgidum* ssp. *dicoccoides* transferred to bread wheat. Crop Science 40: 518-524.

Khatkar BS, Fido RJ, Tatham AS, Schofield JD (2002) Functional properties of wheat gliadins. I. Effects on mixing characteristics and bread making quality. Journal of Cereal Science 35: 299-306.

Khattak AB, Jabbar A, Khan M, Bibi N, Chaudry MA, Khattak MS (2005) Evaluation of physical and chemical characteristics of newly evolved wheat cultivars. Journal of the Science of Food and Agriculture 85: 1061-1064.

Khodos VN, Pshenichnaya AK, Vakula TM, Foya MI (1987) Physiological and genetic aspects of protein accumulation in the caryopses of wheat. Fiziologiya i Biokimiya Kul'turnykh Rastenii 19: 41-45.

Khush GS (2002) Molecular genetics-plant breeder's perspective. In: Mohan Jain S, Brar DS, Ahloowalia BS (eds.) Molecular Techniques in Crop Improvement. Kluwer Academic Publisher. Dodrecht, The Netherlands. P: 1-8.

Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J (2007) In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilization to the grain correlates with agronomic and nitrogen physiological markers. Field Crop Research 102: 22-32.

Kilian A, Kleinhofs A, Warner RL (1992) Localization of NAD(P)H-bispecific nitrate reductase genes to chromosomes of barley, wheat, and Aegilops umbellulata. Theoretical and Applied Genetics 85: 274-275.

Knapp SJ, WW Stroup, WM Ross (1985) Exact confidence intervals for heritability on progeny means basis. Crop Science 25: 192-194.

Knott DR (1995) Durum wheat. In: Slinkard AE, Knott DR (eds.) Harvest of God: The history of field crop breeding in Canada. Saskatoon, Canada: University Extension Press, University of Saskatchewan, Saskatoon. P: 67-81.

Knox RE, Houshmand S, Clarke FR, Clarke JM, Ames NA (2004) QTL association with measures of gluten strength across environments in durum wheats. In: Lafiandra D, Masci S, D'Odivio R (eds.). The Gluten Protein. The proceedings of the 8th Gluten Workshop held on 8-10 September 2003 in Viterbo, Italy. Royal Society of Chemistry. Great Britain. 2004. P: 148-151.

Knox RE, Clarke JM, DePauw RM (2000) Dicamba and growth condition effects on doubled haploid production in durum wheat crossed with maize. Plant Breeding 119: 289-298.

Kondrat'-ev MN, Kamalova TG (1983) Leaf protease in plant ontogenesis. Fiziologiya i Biokimiya Kul'turnykh Rastenii 15: 107-115.

Korzun V, Röder MS, Wendehake K, Pasqualone A, Lotti C, Ganal MW, Blanco A (1999) Integration of dinucleotide microsatellites from hexaploid bread wheat

into a genetic linkage map of durum wheat. Theoretical and Applied Genetics 98: 1202-1207.

Koutroubas SD, Papakosta DK, Doitsinis A (2004) Cultivar and seasonal effects on the contribution of pre-anthesis assimilates to safflower yield. Field Crops Research 90: 263-274.

Kovacs MIP, Howes, Clarke JM, Leisle D (1998) Quality characteristics of durum wheat lines deriving high protein from *Triticum dicoccoides* (6B) substitution. Journal of Cereal Science 27: 47-51.

Lafiandra D, Kasarda DD, Moris R (1984) Chromosomal assignment of gene coding for the wheat gliadin protein components of the cultivar Cheyenne and Chinese Spring by teo dimensional electrophoresis. Theoretical and Applied Genetics 68: 531-539.

Lalonde S, Wipf D, Frommer WB (2004) Transport mechanism for organic forms of carbon and nitrogen between source and sink. Annual Review of Plant Biology 55: 341-372.

Lea PJ, Robinson SA, Steward GR (1990) The enzymology and metabolism of glutamine, glutamate and asparagine. In: B.J. Miflin, P.J. Lea (eds). The Biochemistry of Plants: Amino Acids and Derivatives. Vol. 16. Academic Press, New York. P: 121-159.

Lea PJ, Miflin BJ (1980) Transport and metabolism of asparagine and other nitrogen compounds within the plants. In: Stumpt PK, Conn EE (eds.) The Biochemistry of Plants. Vol. 5. Academic Press, New York. P: 569-607.

Legge WG, Leisle D, McVetty PBE, Evans LE (1991) Effectiveness of two methods of early generation selection for protein content in durum wheat. Canadian Journal of Plant Science 71: 629-640.

Levy AA, Feldman M (1989) Location of genes for high grain protein percentage and other quantitative traits in wild wheat *Triticum turgidum* var. *dicoccoides* Euphytica 41: 113-122.

Loffler CM, Busch RH (1982) Selection for grain protein, grain yield, and nitrogen partitioning efficiency in hard red spring wheat. Crop Science 22: 211-226.

Long DS, Engel RE, Siemens MC (2008) Measuring grain protein concentration with in-line near infrared reflectance spectroscopy. Agronomy Journal 100: 247-252.

Maccaferri M, Sanguineti MC, Donini P, Tuberosa R (2003) Microsatellite analysis reveals a progressive widening of the genetic basis in the elite durum wheat germplasm. Theoretical and Applied Genetics 107: 783-797.

MacRitchie F (1999) Wheat proteins: characterization and role in flour functionality. Cereal Foods World 44: 188-193.

Mae T (1997) Physiological nitrogen efficiency in rice: nitrogen utilization, photosynthesis, and yield potential. Plant and Soil 196: 201-210.

Marchylo BA, Dexter JE, Clarke JM, Ames N (1998) Effects of protein content on CWAD quality. In: Fowler DB, Geddes WE, Johnston AM, Preston KR (eds.) Wheat protein production and marketing. Proc. of the Wheat Protein Symposium. March 9 and 10, 1998. Univ. Extension Press. Univ. of Saskatchewan, Saskatoon, Canada. P: 53-62

Martin de Molino IM, Martinez-Carrasco R, Perez P, Hernandez L, Morcuende L, Sanches de la Puente L (1995) Influence of nitrogen supply and sink strength on changes in leaf nitrogen compounds during senescence in two wheat cultivars. Physiologia Plantarum 95:51-58.

Matus-Cadiz, Pozniak CJ, Hucl PJ (2008) Puroindoline allele diversity in Canadian and Northern US hard spring wheat varieties differing in kernel hardness. Canadian Journal of Plant Science 88: 873-882.

McCaig TN, McLeod JG, Clarke JM, DePauw RM (1992) Measurement of durum pigment with an NIR instrument operating in the visible range. Cereal Chemistry 69: 671-672.

McCartney CA, Somers DJ, Humphreys DG, Lukow O, Ames N, Noll J, Cloutier S, McCallum BD (2005) Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452 × 'AC Domain'. Genome 48: 870-883.

McClung AM, Cantrell RG, Quick JS, Gregory RS (1986) Influence of the *Rht1* semidwarf gene on yield, yield components, and grain protein in durum wheat. Crop Science 26: 1095-1099.

McNeal FH, Berg MA, McGuire CF, Stewart VR, Baldrige DE (1972) Grain and plant nitrogen relationships in eight spring wheat crosses *Triticum aestivum.* Crop Science 12: 599-602.

Mercier S, Hyberg B (1995) Grain quality revisited. American Agricultural Economics Association, Choises 1: 35-38.

Mesfin A, Frohberg RC, Anderson JA (1999) RFLP markers associated with high grain protein from *Triticum turgidum* L. var. *dicoccoides* introgressed into hard red spring wheat. Crop Science 39: 508-513.

Metakovsky EV, Branlard G (1998) Genetic diversity of French common wheat germplasm based on gliadin alleles. Theoretical and Applied Genetics 96: 209-218.

Mickelson S, See D, Meyer FD, Garner JP, Foster CR, Blake TK, Fischer AM (2003) Mapping of QTL associated with nitrogen storage and remobilization in barley (*Hordeum vulgare* L.) leaves. Journal of Experimental Botany 54: 801-812.

Miflin BJ, Habash DZ (2002) The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. Journal of Experimental Botany 53: 979-87.

Millet E, Zaccai M, Feldman M (1992) Paternal and maternal effects on grain wheat and protein percentages in crosses between hexaploid and tetraploid high protein and low protein wheat genotypes. Genome 35: 257-60.

Mitra R, Bhatia CR (1973*)* Studies on protein biosynthesis in developing wheat kernels. In: FAO/IAEA. Nuclear Techniques for Seed Protein Improvement. P: 379-389.

Motalebi, M, Keshawarzi M, Naghavi MR (2007) Glutenin subunit composition in durum (*Triticum durum*) landraces and cultivars. Asian Journal of Plant Sciences 6: 399-402.

Morris CF (2002) Puroindolines: the molecular genetic basis of wheat grain hardness. Plant Molecular Biology. 48: 633-647.

Muller S, Wieser H (1995) Disulphide bonds of gamma-type gliadins. Journal of Cereal Science 22: 21-27.

Muller S, Wieser H (1997) The location of disulphide bonds in monomeric γ-gliadins. Journal of Cereal Science 26: 169-176.

Muurinen S, Kleemola J, Peltonen-Sainio P (2007) Accumulation and translocation of nitrogen in spring cereal cultivars differing in nitrogen use efficiency. Agron Journal 99: 442-449.

Nachit MM, Elouafi I, Pagnotta A, El Saleh A, Iacono E, Labhilili M, Asbati A, Azrak M, Hazzam H, Benscher D, Khairallah M, Ribaut J-M, Tanzarella OA, Porceddu E , Sorrells ME (2001) Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L. var. *durum*). Theoretical and Applied Genetics 102: 177-186.

Nachit MM, Sorrels M, Labhilili M, Baum M, Asbati A, Azrak M, Baalabaki M (1995) Durum wheat improvement, grain quality. Cereal Improvement Program. Ann. Report. P: 102-105.

Ng PKW, Slominski E, Johnson WT (1991) Changes in wheat endosperm proteins during grain maturation. In: Bushuk W, Tkachuk R (eds.) Gluten protein 1990. AACC. St Paul, Minnesota, USA. P: 740-754.

Norman RJ, Guindo D, Wells BR, Wilson CE (1992) Seasonal accumulation and partitioning of nitrogen^{-15} in rice. Soil Science Society of America Journal 56: 1521-1527.

Novaro P, D'Egidio MG, Mariani BM, Nardi S (1993) Combined effect of protein content and high-temperature drying system on pasta cooking quality. Cereal Chemistry 70: 716-719.

Ntanos DA, Koutroubas SD (2002) Dry matter and N accumulation and translocation for Indica and Japonica rice under Mediterranean conditions. Field Crops Research 74: 93-101.

Olmos S, Distelfeld A, Chicaiza O, Schlatter AR, Fahima T, Echenique V, Dubcovsky J (2003) Precise mapping of a locus affecting grain protein content in durum wheat. Theoretical and Applied Genetics 107: 1243-1251.

Østergaard H, Rasmussen SK, Roberst TH, Hejgaard J (2000) Inhibitory serpins from wheat grain with reactive centers resembling glutamine-rich repeats of prolamin storage proteins. Journal of Biological Chemistry 275: 33272-33279.

Pagnotta MA, Blanco A, Gadaleta A, Fares C (2005) Functional determinants of grain quality. In: Royo C, Nachit MM, Di Fonzo N, Araus JL, Pfeiffer WH, Slafer GA (eds.). 2005. Durum wheat breeding. Current approaches and future strategies. Vol.2. P: 483-511.

Papakosta DK, Garianas AA (1991) Nitrogen and dry matter accumulation, remobilization, and losses for mediterranean wheat during grain filling. Agronomy Journal 83: 864-870.

Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. Journal of the Science Food Agriculture 40: 51-65.

Payne PI, Jackson EA, Holt LM (1984) The association between γ-gliadin 45 and gluten strength in durum wheat varieties. A direct causal effect on the result of genetic linkage. Journal of Cereal Science 2: 73-81.

Payne PI, Holt LM, Lawrence GJ (1983) Detection of a novel high molecular weight subunit of glutenin in some Japanese hexaploid wheats. Journal of Cereal Science 1: 3-8

Peeters KMU, Van Laere AJ (1994) Amino acid metabolism associated with N-mobilization from the flag leaf of wheat (*Triticum aestivum* L.) during grain development. Plant, Cell and Environment 17: 131-141.

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400: 256-261.

Peoples MB, Dalling MJ (1988) The interplay between proteolysis and amino acid metabolism during senescence and nitrogen reallocation. In: Nooden LD, Leopold AC (eds.). Senescence and Aging in Plants. San Diego. Academic Press. P: 181-217.

Perretant MR, Cadalen T, Charmet G, Sourdille P, Nicolas P, Boeuf C, Tixier MH, Branlanrd G, Bernard S, Bernard M (2000) QTL analysis of bread-making quality in wheat using a double haploid population. Theoretical and Applied Genetics 100: 1167-1175.

Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific to the D genome of bread wheat. Genome 43: 689-697.

Pinthus MJ, Gale MD (1990) The effects of 'gibberellin-insensitive' dwarfing alleles in wheat on grain weight and protein content. Theoretical and Applied Genetics 79: 108-112.

Pogna NE, Redaelli R, Dachkevitch T, Curioni A, Dalbelin Deruffo A (1994) Genetics of wheat quality and its improvement by conventional and biotechnological breeding. in: Bushuk W, Rasper VF (eds.) Wheat-Production, Properties and Quality. Blackie Academic and Professional, Glasgow, UK. P: 205-224

Pogna NE, Autran JC, Mellini F, Lafiandra D, Feillet P (1990) Chromosome B coded gliadins and glutenin subunits in durum wheat: Genetics and relationship to gluten strength. Journal of Cereal Science 11: 15-34.

Pogna NE, Boggini G, Corbellini M, Cattaneo M, Dal Belin Peruffo A (1982) Association between gliadin electrophoretic bands and quality in common wheat. Canadian Journal of Plant Science 62: 913-918.

Pozniak CJ, Fox L, Knot DR (2009) CDC Verona durum wheat. Canadian Journal of Plant Science 89: 321-324.

Pozniak CJ, Knox RE, Clarke FR, Clark JM (2007) Identification of QTL and association of a phytoene synthase gene with endosperm colour in durum wheat. Theoretical and Applied Genetics 114: 525-537.

Prasad M, Kumar N, Kulwal PL, Röder M, Balyan HS, Dhaliwal HS, Gupta PK (2003) QTL analysis for grain protein content using SSR markers and validation studies using NILs in bread wheat. Theoretical and Applied Genetics 106: 659-667.

Prasad M, Kumar N., Kulwal PL, Röder MS, Balyan HS, Dhaliwal HS, Gupta PK (2002) QTL analysis for grain protein content using SSR markers and validation studies using NILs in bread wheat. Theoretical and Applied Genetics Springer-Verlag 2002. DOI 10.1007/s0012-002-1114-y.

Prasad M, Varshney RK, Kumar A, Balyan HS, Sharma PC, Edwards KJ, Singh H, Dhaliwal HS, Roy JK, Gupta PK (1999) A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. Theoretical and Applied Genetics 99: 341-345.

Quesada A, Krapp A, Trueman LJ, Danielvedele F, Fernandez E, Forde BG, Caboche M (1997) PCR-identification of a *Nicotiana plumbaginifolia* cDNA homologous to the high-affinity nitrate transporters of the crn family. Plant Molecular Biology 34: 265-274.

Rao BN (2008) A study of the rheological properties and gluten protein components associated with enhanced baking quality in durum wheat (*Triticum turgidum* L. var. *durum*). MSc Thesis. Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada.

Reed AJ, Below FE, Hageman H (1980) Grain protein accumulation and the relationship between leaf nitrate reductase and protease activities during grain development in maize (*Zea mays* L.). Variation between genotypes. Plant Physiology 66: 164-170.

Rebetzke GJ, Richards RA, Fischer VM, Mickelson BJ (1999) Breeding long coleoptile, reduced height wheats. Euphytica 106: 159-168.

Reimer S, Pozniak CJ, Clarke FR, Clarke JM, Somers DJ, Knox RE, Singh AK (2008) Association mapping of yellow pigment in an elite collection of durum wheat cultivars and breeding lines. Genome 51: 1016-1025.

Rentsch D, Schmidt S, Tegeder M (2007) Transporters for uptake and allocation of organic nitrogen compounds in plants. Federation of European Biological Societies (FEBS) Letters 581: 2281-2289

Rharrabti Y, Villegas D, Garcia de Moral LF, Aparicio N, Elhani S, Royo C (2001) Environmental and genetic determination of protein content and grain yield in durum wheat under Mediterranean conditions. Plant Breeding 120: 381-388.

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Gamal MW (1998) A microsatellite map of wheat. Genetics 149: 2007-2023.

Sakakibara H, Watanabe M, Hase T, Sugiyama T (1991) Molecular cloning and characterization of complementary DNA encoding for ferredoxin-dependent glutamate synthase in maize leaf. J Biol Chem 266: 2028-2035

Sapirstein H, Fu B (1998) Intercultivar variation in the quantity of monomeric proteins, soluble and insoluble glutenin, and residue protein in wheat flour and relationships to breadmaking quality. Cereal Chemistry 74: 500-507.

SAS Institute Inc. (2003) SAS User's Guide. Version 8. SAS Institute, Inc., Carry New York.

Schnorr KM, Juricek M, Huang CX, Culley D, Kleinhofs A (1991) Analysis of barley nitrate reductase cDNA and genomic clones. Molecular and General Genetics 227: 411-416

Schofield JD (1994) Wheat proteins; structure and functionality in milling and breadmaking. In: Bushuk W, Rasper VF (eds.) Wheat production, properties and quality. Blackie Academie & Professional. Chapman & Hall. Cambrige University Press, Great Britain. P: 73-106.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18: 233-234.

See D, Kanazin V, Kephart K, Blake T (2002) Mapping genes controlling variation in barley grain protein concentration. Crop Science 42: 68-685.

Sharma D, Knott DR (1964) The inheritance of seed weight in a wheat cross. Canadian Journal of Genetics and Cytology 6: 419-425.

Sheehy JE, Mnzava M, Cassman KG, Mitchell PL, Pablico P, Robles RP, Samonte HP, Lales JS, Ferrer AB (2004) Temporal origin of nitrogen in the grain of irrigated rice in the dry season: The outcome of uptake, cycling, senescence and competition studied using a 15N-point placement technique. Field Crops Research 89: 337-348.

Shewry PR, Halford NG (2002) Cereal seed storage proteins: structures, properties and role in grain utilization. Journal of Experimental Botany 53: 947-958.

Shewry PR, Tatham AS, Barro F, Barcelo P, Lazzeri P (1995) Biotechnology of bread-making: unraveling and manipulating the multi-protein gluten complex. Biotechnology 13: 1185-1190.

Shewry PR, Tatham AS (1990) The prolamin storage proteins of cereal seeds: structure and evolution. Journal of Biochemistry 267: 1-12.

Shewry PR, Tatham AS, Forde J, Kreis M, Miflin BJ (1986) The classification and nomenclature of wheat gluten proteins: A reassessment. Journal of Cereal Science 4: 97-106.

Shewry PR, Lafiandra D, Salsedo G, Aragoncillo C, Garcia-Omedo F, Lew EJL, Dietler MD, Kasarda DD (1984) N-terminal amino acid sequences of chloroform/methanol-soluble proteins and albumins from endosperms of wheat, barley and related species. Federation of European Biological Societies (FEBS) Letters 175: 355-363.

Sieciechowicz KA, Joy KW, Ireland RJ (1988) The metabolism of asparagine in plants. Phytochemistry 27: 663-671.

Simmonds DH (1978) Structure, composition and biochemistry of cereal grains. In: Pomeranz Y (ed.) Cereals '78: Better Nutrition for the World's Millions. Am. Assoc. Cereal Chem. St Paul. MN. P: 105.

Sinclair TR, Pinter PJ, Kimball BA, Adamsen FJ, LaMorte RL, Wall GW, Hunsaker DJ, Adam N, Brook TJ, Garcia RL, Thompson T, Leavitt S, Mattias A (2000) Leaf nitrogen concentration of wheat subjected to elevated [CO2] and either water or N deficits. Agriculture, Ecosystems & Environment 79: 53-60.

Singh H, MacRitchie F (2001) Application of polymer science to properties of gluten. Journal of Cereal Science 33: 231-243.

Singh A, Reimer S, Pozniak CJ, Clarke JM, Knox RE, Singh AK (2009) Allelic variation at Psy1-A1 and association with yellow pigment in durum wheat grain. Theoretical and Applied Genetics (in press).

Smalle J, Vierstra RD (2004) The ubiquitin 26S proteasome proteolytic pathway. Annual Review of Plant Biology 55: 555-590.

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 109: 1105-1114.

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theoretical and Applied Genetics 110: 550-560

Sourdille P, Cadalen T, Guyonarc'h H, Snape JW, Perretant MR, Charmet G, Boeuf C, Bernard S, Bernard M (2003) An update of the Courtot x Chinese Spring intervarietal molecular marker linkage map for QTL detection of agronomic traits in wheat. Theoretical and Applied Genetics 106: 530-538.

Steiger DK, Elias EM, Cantrell RG (1996) Evaluation of lines derived from wild emmer chromosome substitutions: I. Quality traits. Crop Science 36: 223-227.

Stoddart FL, Marshall DR (1990) Variability in grain protein in Australian hexaploid wheats. Australian Journal of Agricultural Research 41: 277-288.

Suzuki A, Rothstein S (1997) Structure and regulation of ferredoxin-dependent glutamase synthase from Arabidopsis thaliana. Cloning of cDNA expression in different tissues of wild-type and gltS mutant strains, and light induction. European Journal of Biochemistry 243: 708-718.

Tahir ISA, Nakata N (2005) Remobilization of nitrogen and carbohydrate from stems of bread wheat in response to heat stress during grain filling. Journal of Agronomy and Crop Science 191: 106-115.

Tatham AS, Drake AF, Shewry PR (1990) Conformational studies of synthetic peptides corresponding to the repetitive region of the high molecular weight (HMW) glutenin subunits of wheat. Journal of Cereal Science 11: 189-200.

Tatham AS, Shewry PR (1995) The S-poor prolamins of wheat, barley and rye. Journal of Cereal Science 22: 1-16.

Townley-Smith TF, DePauw RM, Lendrum CWB, McCrystal GE, Patterson LA (1987) Kyle durum wheat. Canadian Journal of Plant Science 67: 225-227.

Tranquilli G, Heaton J, Chicaiza O, Dubcovsky J (2002) Substitutions and deletions of genes related to grain hardness and their effect on grain texture. Crop Science 42: 1812-1817.

Tranquilli G, Lijavetzky D, Muzzi G, Dubcovsky J (1999) Genetic and Physical characterization of grain texture-related loci in diploid wheat. Molecular and General Genetic 262: 846-850.

Triboï E, Abad A, Michelena A, Lloveras J, Ollier JL, Daniel C (2000) Environmental effects on the quality of two wheat genotypes: 1. quantitative and qualitative variation of storage protein. European Journal of Agronomy 13: 47-64.

Troccoli A, Borreli GM, De Vita P, Fares C, Di Fonzo N (2000) Durum wheat quality: a multidisciplinary concept. Journal of Cereal Science 32: 99-113.

Trueman LJ, Richardson A, Forde BG (1996) Molecular cloning of higher plant homologues of the high-affinity nitrate transporters of *Chlamydomonas reinhardtii* and *Aspergillus nidulans*. Gene 175: 223-231.

Turnbull KM, Turner M, Mukai Y, Yamamoto M, Morell MK, Appels R, Rahman S (2003) The organization of genes tightly linked to the *Ha* locus in *Aegilops taushii*, the D-genome donor to wheat. Genome 46: 330-338.

Turner AS, Bradburne RP, Fish L, Snape JW (2004) New quantitative trait loci influencing grain texture and protein content in bread wheat. Journal of Cereal Science 40: 51-60.

Uauy C, Distelfeld A, Fahima T, Blechi A, Dubcovsky J (2006a) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314: 1298-1301.

Uauy C, Brevis JC, Dubcovsky J (2006b) The high grain protein content gene *Gpc-B1* accelerates senescence and has pleiotropic effects on protein content in wheat. Journal of Experimental Botany 57: 2785-2794.

Vallega V (1985) Identification of a major protein gene compatible with high grain yields in semidwarf *Triticum durum* genotypes. Cereal Research Communications 13: 201-20.

van Ooijen JW, Voorrips RE (2004) JoinMap Version 3.0, software for the calculation of genetic linkage maps. Kyazma BV. Wageningen.

van Ooijen JW (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83: 613-624.

van Sanford DA, Mackown CT (1987) Cultivar differences in nitrogen remobilization during grain filling in soft red winter wheat. Crop Science 27: 295-300.

Vazquez JF, Ruiz M, Nieto-Taladriz MT, Albuquerque MM (1996) Effects on gluten strength of lowM_rGlutenin subunits coded by alleles at *Glu-A3* and *Glu-B3* loci in durum wheat. Journal of Cereal Science 24: 125-130.

Vidmar JJ, Zhuo DeGen, Siddiqi MY (2000) Regulation of high-affinity nitrate transporter genes and high-affinity nitrate influx by nitrogen pools in roots of barley. Plant Physiology 123: 307-318.

Vierstra RD (1996) Proteolysis in plants: mechanisms and functions. Plant Molecular Biology 32: 275-302.

Wang H, McCaig TN, DePauw RM, Clarke FR, Clarke JM (2003) Physiological characteristics of recent Canada western red spring wheat cultivars: components of grain nitrogen yield. Canadian Journal of Plant Science 83: 699-707.

Weegels PL, van de Pijpekamp AM, Graveland A, Hamer RJ, Schofield JD (1996) Depolymerisation and re-polymerisation of wheat glutenin during dough processing. I. Relationships between glutenin macropolymer content and quality parameters. Journal of Cereal Science 23: 103-111.

Weiland RT, Ta TC (1992) Allocation and retranslocation of 15N by maize (*Zea mays* L.) hybrids under field conditions of low and high N fertility. Australian Journal of Plant Physiology 19: 77-88.

Wieser H (2007) Chemistry of gluten proteins. Food Microbiology. 24: 115-119.

Williams P, Sobering D, Antoniszyn J (1998) Protein testing methods. In: Fowler DB, Geddes WE, Johnston AM, Preston KR (eds.) Wheat protein production and marketing. Proc. of the Wheat Protein Symposium. March 9 and 10, 1998. Univ. Extension Press. Univ. of Saskatchewan, Saskatoon, Canada. P: 37-47.

Wilkinson JQ, Crawford NM (1993) Identification and characterization of a chlorate-resistant mutant of *Arabidopsis thaliana* with mutations in both nitrate reductase structural genes NIA1 and NIA2. Molecular General Genetics 239: 289-297.

Winter H, Lohaus G, Heldt HW (1992) Phloem transport of amino acids and sucrose depending on the corresponding metabolite levels in the leaves of barley. Plant Physiology 99: 996-1004

Wittenberg AH, Lee TV, Cayla C, Kilian A, Visser RG, Schouten HJ (2005) Validation of the high-throughput marker technology DArT using the model plant *Arabidopsis thaliana*. Molecular Genetics and Genomics 274: 30-39.

Wrigley CW, Robbinson PJ, Williams WT (1981) Association between electrophoretic patterns of gliadin proteins and quality characteristics of wheat cultivars. Journal of Science Food and Agriculture 23: 433-442.

Zadocks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Zanetti S, Winzeler M, Feulillet C, Keller B, Messmer M (2001) Genetic analysis of bread-making quality in wheat and spelt. Plant Breeding 120: 13-19.

Zanetti S, Keler M, Winzeler M, Saurer W, Keller B, Messmer M (1999) QTL for quality parameters for bread-making in a segregating wheat by spelt populations, In: Genetics and breeding for quality and resistance. Proc. XV. EUCARPIA Congress, Viterbo, Italy, 20-25 September 1998. Kluwer Acad. Pub., Dordrecht, Germany: 357-360.

Zhang W, Dubcovsky J (2008) Association between allelic variation at the *Phytoene synthase 1* gene and yellow pigment content in the wheat grain. Theoretical and Applied Genetics 116: 635-645.

Zhang W, Lukaszewski A, Kolmer J, Soria M, Goyal S, Dubcovsky J (2005) Molecular characterization of durum and common wheat recombinant lines carrying leaf rust resistance (Lr19) and yellow pigment (Y) genes from *Lophopyrum ponticum*. Theoretical and Applied Genetics 111: 573-582.

Zhu Yan, Li Weiguo, Jing Qi, Cao Weixing, Takeshi Horie (2007) Modeling grain protein formation in relation to nitrogen uptake and remobilization in rice plant. Front. Agric. China 2007, 1(1): 8–16. DOI 10.1007/s11703-007-0002-2. Higher Education Press and Springer-Verlag 2007.

Zhuo D, Okamoto M, Vidmar JJ, Glass ADM (1999) Regulation of a putative high-affinity nitrate transporter (*Nrt2;1At*) in roots of *Arabidobsis thaliana*. Plant Journal 17: 563-568.

APPENDICES

Appendix 3.1. Mixed model analysis of the GxE effect on combined data of GPC and grain yield of the DT695 x Strongfield mapping population over six growing environments.

Variables	Effect	Covariance parameter	F value	Variance estimate	Z value ‡
GPC	Entry		3.24 **	-	-
	-	Location	-	2.3428	1.5 ns
	-	Rep (loc)	-	0.2425	1.71 ns
	-	Location*entry	-	0.1219	5.91 **
	-	Residual	-	0.5552	2.36 **
Grain yield	Entry		3.27 **		
	-	Location		2368776	0.16 ns
	-	Rep (loc)		242239	0.17 ns
	-	Location*entry		42644	6.03 **
	-	Residual		0.99	0.99 ns

‡ Z value = standard deviation of sample's data from the population mean
** = significant at the level of 0.01 of probability
ns = not significant

Appendix 3.2. Procedure mixed analysis for GPC data of the DT695 x Strongfield mapping population at each location.

Location	Effect	Covariance parameter	F value	Variance estimate
SC 2002 †	entry		3.16 **	-
		Rep	-	0.11
		Block(rep)	-	0.19
		Residual	-	0.21
RG 2002	entry		3.14 **	-
		Rep	-	0.50
		Block(rep)	-	0.98
		Residual	-	0.32
ST 2003	entry		4.29 **	-
		Rep	-	0.01
		Block(rep)	-	0.13
		Residual	-	0.15
SC 2003	entry		3.17 **	-
		Rep	-	0.03
		Block(rep)	-	0.11
		Residual	-	0.16
RG 2003	entry		4.76 **	-
		Rep	-	0.67
		Block(rep)	-	0.88
		Residual	-	0.20
ST 2005	entry		3.62 **	-
		Rep	-	0
		Block(rep)	-	0.04
		Residual	-	0.18

†RG = Regina, ST = Saskatoon, SC = Swift Current
** = significant at the level of 0.01 of probability

Appendix 3.3. Shapiro-Wilk test for normality for least square means of GPC of the DT695 x Strongfield mapping population over six environments

Location	Statistic		p Value	
SC 2002 †	W	0.99311	Pr < W	0.6288
RG 2002	W	0.990962	Pr < W	0.4002
ST 2003	W	0.987253	Pr < W	0.1099
SC 2003	W	0.987786	Pr < W	0.1134
RG 2003	W	0.988678	Pr < W	0.1477
ST 2005	W	0.993664	Pr < W	0.6118

†RG = Regina, ST = Saskatoon, SC = Swift Current
Shapiro-Wilk test with (Pr < W) is larger than 0.05 means the data are normally distributed.

Appendix 3.4. Procedure mixed analysis for GPC data of the 9370-DJ**3 x Strongfield validation population at each location.

Location	Effect	Covariance parameter	F value	Variance estimate
SC 2002 †	entry		6.10 **	-
		Rep	-	0.04
		Block(rep)	-	0.20
		Residual	-	0.18
RG 2002	entry		5.06	-
		Rep	-	0.01
		Block(rep)	-	0.07
		Residual	-	0.14
ST 2003	entry		3.26	-
		Rep	-	0
		Block(rep)	-	0.16
		Residual	-	0.31
SC 2003	entry		9.78	-
		Rep	-	0.42
		Block(rep)	-	0.28
		Residual	-	0.17
RG 2003	entry		16.33	-
		Rep	-	0
		Block(rep)	-	0.13
		Residual	-	0.08

† RG = Regina, ST = Saskatoon, SC = Swift Current
** = significant at the level of 0.01 of probability

Appendix 3.5. Mixed model analysis of the GxE effect on combined data of GPC of the 9370-DJ**3 x Strongfield validation population over six environments.

Effect	Covariance parameter	F value	Variance estimate	Z value ‡
Entry	-	5.38 **	-	-
-	Location	-	5.27	1.40 ns
-	Rep (loc)	-	0.10	1.53 ns
-	Location*entry	-	0.22	7.67 **
-	Residual	-	0.33	16.11 **

‡ Z = standard deviation of sample's data from the population mean
** = significant at the level of 0.01 of probability
ns = not significant

Appendix 3.6. Shapiro-Wilk test for normality for least square means of GPC of the 9370-DJ**3 x Strongfield validation population over five environments

Location	Statistic		p Value	
SC 2002 †	W	0.987616	Pr < W	0.4803
RG 2002	W	0.984186	Pr < W	0.2708
SC 2003	W	0.977109	Pr < W	0.0735
RG 2003	W	0.980643	Pr < W	0.1210
ST 2003	W	0.979467	Pr < W	0.0877

† RG = Regina, ST = Saskatoon, SC = Swift Current
Shapiro-Wilk test with (Pr < W) is larger than 0.05 means the data are normally distributed.

Appendix 4.1. SAS output of mixed procedures analysis for combined data of Kernen 2005, Goodale 2006 and Nasser 2006. As each location was intentionally chosen for its soil N level, as such, similar to entry, location was also determined as fixed effect.

Variables	Effect	Covariance parameter	F value	Variance estimate
Plant height	entry		72.35 **	-
	location		996.24 **	-
	entry*loc		3.57 **	-
		block	-	1.11
		residual	-	18.93
Days to heading	entry		38.6 **	-
	location		2695.20 **	-
	entry*loc		2.38 **	-
		block	-	0
		residual	-	0.28
Days to maturity	entry		2.61 **	-
	location		2145.23 **	-
	entry*loc		ns	-
		block	-	0.28
		residual	-	1.79
Thousand kernel weight	entry		5.90 **	-
	location		95.90 **	-
	entry*loc		1.86 **	-
		block	-	0.05
		residual	-	5.78
TPN-An	entry		3.99 **	-
	location		88.18 **	-
	entry*loc		1.54 *	-
		block	-	0
		residual	-	8.3
TPN-Milk stage	entry		3.42 **	-
	location		92.28 **	-
	entry*loc		2.18 **	-
		block	-	0.25
		residual	-	13.62
TPN-Dough stage	entry		6.25 **	-
	location		189.91 **	-
	entry*loc		ns	-
		block	-	0
		residual	-	11.72
TPN-PM	entry		8.65 **	-
	location		233.04 **	-
	entry*loc		ns	-
		block	-	0
		residual	-	11.63

Appendix 4.1 (continued)

Variables	Effect	Covariance parameter	F value	Variance estimate
VegN-Milk stage	entry		2.80 **	-
	location		274.18 **	-
	entry*loc		ns	-
		block	-	0.42
		residual	-	13.76
VegN-Dough stage	entry		3.10 **	-
	location		281.55 **	-
	entry*loc		ns	-
		block	-	0.55
		residual	-	8.37
VegN-PM	entry		3.06 **	-
	location		188.58 **	-
	entry*loc		ns	-
		block	-	0.44
		residual	-	3.65
GN-Milk stage	entry		7.11 **	-
	location		147.14 **	-
	entry*loc		ns	-
		block	-	0
		residual	-	4.88
GN-Dough stage	entry		3.68 **	-
	location		8.69 **	-
	entry*loc		ns	-
		block	-	0.31
		residual	-	9.33
GN-PM	entry		5.69 **	-
	location		69.07 **	-
	entry*loc		ns	-
		block	-	0.76
		residual	-	8.55
GPC	entry		12.42 **	-
	location		420.73 **	-
	entry*loc		4.09 **	-
		block	-	0.0003
		residual	-	0.41
NHI	entry		3.14 **	-
	location		106.25 **	-
	entry*loc		ns	-
		block	-	2
		residual	-	10.66
Remobilized N (NR)	entry		3.21 **	-
	location		ns	-
	entry*loc		ns	-
		block	-	0.62
		residual	-	7.96

Appendix 4.1 (continued)

Variables	Effect	Covariance parameter	F value	Variance estimate
Post-anthesi N Uptake	entry		4.73 **	-
	location		430.97 **	-
	entry*loc		1.57 *	-
		block	-	0
		residual	-	7.07
Grain N-remobilized (Grain NR)	entry		2.87 **	-
	location		381.12 **	-
	entry*loc		ns	-
		block	-	0.09
		residual	-	56.94
Grain yield	entry		8.36 **	-
	location		1052.42 **	-
	entry*loc		2.04 **	-
		block	-	678
		residual	-	89160
Protein yield	entry		6.82 **	-
	location		1330 **	-
	entry*loc		2.29 **	-
		block	-	20
		residual	-	1903

Appendix 4.2. Least square means of total plant nitrogen (TPN), vegetative nitrogen (VN) and grain nitrogen (GN) among different growth stages.

Locations	**Kernen**			**Goodale**			**Nasser**		
	TPN	**VN**	**GN**	**TPN**	**VN**	**GN**	**TPN**	**VN**	**GN**
Anthesis	39.9	39.9	0	42.6	42.6	0	35.3	35.4	0
Milk	44.5	32.2	12.3	43.2	32.3	10.9	35.5	17.8	17.7
Dough	47.5	25.1	22.5	45.8	20.9	24.9	35.6	12.1	23.5
Maturity	49.5	13.7	34.0	47.2	15.7	31.4	36.3	8.8	27.5
LSD (P<0.05)	1.3	1.0	1.1	1.3	1.4	1.1	1.4	0.8	0.9

Appendix 4.3. Least square means of total plant nitrogen (TPN), vegetative nitrogen (VN) and grain nitrogen (GN) during phenological development at different locations

Locations	TPN1	TPN2	TPN3	TPN4	VN1	VN2	VN3	VN4	GN1	GN2	GN3	GN4
Kernen 2005	39.9	44.5	47.5	49.5	39.9	32.2	25.1	13.7	0	12.3	22.5	34.0
Goodale 2006	42.6	43.2	45.8	47.2	42.6	32.3	20.9	15.7	0	10.9	24.9	31.4
Nasser 2006	35.4	35.5	35.6	36.3	35.4	17.8	12.1	8.8	0	17.8	23.5	27.5
LSD (P<0.05)	1.2	1.4	1.3	1.3	1.2	1.3	1.1	0.7	0	0.8	1.1	2.1
SEM	0.41	0.50	0.47	0.47	0.41	0.47	0.39	0.25	0	0.27	0.4	0.61

TPN1, TPN2, TPN3, TPN4 = total plant nitrogen at anthesis, milk stage, dough and physiological maturity, respectively.
VN1, VN2, VN3, VN4 = nitrogen in the vegetative tissue at anthesis, milk stage, dough and physiological maturity, respectively.
GN1, GN2, GN3, GN4 = grain nitrogen at anthesis, milk stage, dough and physiological maturity, respectively.

Appendix 4.4. Contrast analysis of lower GPC DH and higher GPC DH on variables related to grain N

	F calculated for plant height				F calculated for days to heading				F calculated for days to maturity			
	Kernen	G'dale	Nasser	Mean	Kernen	G'dale	Nasser	Mean	Kernen	G'dale	Nasser	Mean
Lower GPC DH vs higher GPC DH	11.33 **	18.18 **	12.79 **	39.16 **	31.24 **	101.40 **	57.89 **	159.78 **	ns	ns	ns	ns
Strongfield vs higher GPC DH	ns	ns	ns	ns	12.65 **	26.79 **	32.02 **	62.65 **	ns	ns	ns	ns
DT695 vs lower GPC DH	ns	4.40 *	10.53 **	12.91 **	ns	ns	ns	ns	ns	ns	ns	ns

	F calculated for seed weight				F calculated for grain yield				F calculated for protein yield			
	Kernen	G'dale	Nasser	Mean	Kernen	G'dale	Nasser	Mean	Kernen	G'dale	Nasser	Mean
Lower GPC DH vs higher GPC DH	ns	ns	ns	ns	30.48 **	ns	17.78 **	4.23 *	10.92 **	6.15 *	ns	14.27 **
Strongfield vs higher GPC DH	ns	7.94 **	4.97 *	10.96 **	ns	ns	6.41 *	ns	ns	8.8 2 **	ns	ns
DT695 vs lower GPC DH	ns	7.08 *	ns	5.92 *	4.15 *	ns	ns	ns	ns	ns	ns	7.63 **

	F calculated for GPC				F calculated for NHI				F calculated for NR			
	Kernen	G'dale	Nasser	Mean	Kernen	G'dale	Nasser	Mean	Kernen	G'dale	Nasser	Mean
Lower GPC DH vs higher GPC DH	104.21 **	6.80 *	28.18 **	95.49 **	ns	ns	ns	ns	ns	8.92 **	ns	5.49 *
Strongfield vs higher GPC DH	ns	9.08 **	ns	5.26 *	ns	ns	ns	ns	ns	10.52 **	ns	7.02 **
DT695 vs lower GPC DH	6.28 *	16.71 **	ns	ns	ns	ns	4.28 *	4.07 *	ns	ns	ns	ns

Appendix 4.4. (continued)

	F calculated for NUP				F calculated for Seed N remob			
	Kernen	G'dale	Nasser	Mean	Kernen	G'dale	Nasser	Mean
Lower GPC DH vs higher GPC DH	5.87 *	24.53 **	ns	14.91 **	4.82 *	25.54 **	ns	12.11 **
Strongfield vs higher GPC DH	ns	5.91*	ns	4.39 *	ns	ns	ns	ns
DT695 vs lower GPC DH	**	**	**	*	ns	ns	ns	ns

	F calculated for TPN1				F calculated for TPN4				F calculated for GN4			
	Kernen	G'dale	Nasser	Mean	Kernen	G'dale	Nasser	Mean	Kernen	G'dale	Nasser	Mean
Lower GPC DH vs higher GPC DH	ns	ns	ns	ns	ns	ns	4.86 *	5.76 *	ns	ns	12.57 **	ns
Strongfield vs higher GPC DH	ns	7.87 **	ns	9.97 **	ns	ns	ns	5.56 *	ns	ns	ns	6.17 *
DT695 vs lower GPC DH	ns	ns	ns	6.43 *	ns	6.95 *	4.67 *	13.20 **	ns	6.96 *	ns	6.44 *