Governing Grain Protein Concentration and Composition in Wheat and Barley: Use of Genetic and Environmental factors

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Cover: wheat spike (left) and barley spike (right). Pictures were taken in barley and wheat fields in Alnarp, Sweden.
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Abstract

For commercial production of wheat and barley, grain yield (GY), grain protein concentration (GPC) and protein composition are considered important. Genetic (G) and environmental (E) factors are important constraints affecting GPC and protein composition in wheat and barley. This thesis examined the options to govern GPC and protein composition in wheat and barley grain by using G and E factors. The results showed that combination of G and E factors played an important role, more important than single factors solely, to determine GPC, protein composition, accumulation and protein breakdown in wheat and barley grains. Differences in maturation times of wheat and barley plants, due to variation in G and E factors, were found to be a significant factor in determining GPC and protein composition. By governing the maturation times, using different genotypes, N application rates and timings, pre- and post-anthesis temperature, the options to govern GPC and protein composition increased. Early maturing cultivars, N application at anthesis and high pre-anthesis temperature resulted in high amounts of sodium dodecyl sulphate (SDS)-extractable proteins (TOTE). Late maturing cultivars, N application at spike formation and high post-anthesis temperature resulted in high percentage of SDS- unextractable polymers into total polymers (%UPP). Pre-anthesis temperature influenced mainly TOTE, while post-anthesis temperature influenced mainly %UPP. Maturation time was found more important for determining GPC and protein composition at high temperature while at low temperature late nitrogen supply was of higher relevance. Differences in the build-up of TOTE and polymeric proteins were found to initiate from 12 days after anthesis and thereafter the build-up rate pertained throughout the grain maturation period. In barley, breakdown of proteins at malting were found dependent on plant maturation time and GPC i.e. higher breakdown rate at higher GPC. A negative correlation was found between GY and TOTE and between TOTE and %UPP. The results from this thesis help to understand how GPC, protein composition, accumulation and breakdown are governed in wheat and barley by various G and E factors. Moreover, the results may help in creating a simulation based quality model in which both G and E factors can be used to model GPC and protein composition in wheat and barley.

Keywords: End-use quality, grain filling period, *Hordeum vulgare*, maturation time, time to flowering, *Triticum aestivum*,

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Dedication

To my mother ***Gulnar Andleeb*** and father ***Hafeez Ahmed Malik***

Almighty Allah said:

''Whoever is given wisdom and knowledge is blessed with bounties in good abundance" (**Surah al-Baqarah, 2:272**)

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ali Hafeez Malik, Allan Andersson, Ramune Kuktaite, Muhammad Yaqub Mujahid, Bismillah Khan and Eva Johansson (2012). Genotypic variation in dry weight and nitrogen concentration of wheat plant parts; relations to grain yield and grain protein concentration. *(Manuscript)*.

II Ali Hafeez Malik, Lena Holm and Eva Johansson (2012). Soil and starter fertiliser affect yield and protein composition of malting barley. *(Submitted)*.

III Ali Hafeez Malik, Maria Luisa Preito-Linde, Ramune Kuktaite, Allan Andersson and Eva Johansson (2011). Individual and interactive effects of genetic background and environmental conditions on amount and size distribution of polymeric proteins in wheat grain. *Czech Journal of Genetics and Plant Breeding* 47: S186-S189.

IV Ali Hafeez Malik, Ramune Kuktaite and Allan Andersson. Individual and combined effect of pre- and post-anthesis temperature on grain yield and protein composition of two malting barley cultivars. *(Manuscript)*

V Ali Hafeez Malik, Maria Luisa Preito-Linde, Ramune Kuktaite, Allan Andersson and Eva Johansson (2011). Individual and interactive effects of cultivar maturation time, nitrogen regime and temperature level on accumulation of wheat grain proteins. *Journal of the Science of Food and Agriculture* 91: 2192-2200.

VI Ali Hafeez Malik, Lena Holm and Eva Johansson (2012). Governing plant development in barley: Relation to protein composition and break-down rates of the protein polymers during malting *(Manuscript)*.

VII Ali Hafeez Malik, Ramune Kuktaite and Eva Johansson (2012). Accumulation of proteins in the wheat grain: The combined effect of genetic and environmental factors and relation to bread-making quality. *(Submitted)*.

Papers III and V are reproduced with the permission of the publishers.

The contribution of Ali Hafeez Malik to the papers included in this thesis was as follows:

I Planned the experiment with supervisors, performed all experimental and practical work, evaluated and analysed the data and wrote the manuscript in collaboration with the co-authors.

II Planned the experiment with supervisors, performed all experimental and practical work with one coauthor, evaluated and analysed the data and wrote the manuscript in collaboration with the co-authors.

III Planned the experiment with supervisors, performed all experimental and practical work, evaluated and analysed the data and wrote the manuscript in collaboration with the co-authors.

IV Evaluated and analysed the data and wrote the manuscript in collaboration with the co-authors.

V Planned the experiment with supervisors, performed all experimental and practical work, evaluated and analysed the data and wrote the manuscript in collaboration with the co-authors.

VI Planned the experiment with supervisors, performed all experimental and practical work, evaluated and analysed the data and wrote the manuscript in collaboration with the co-authors.

VII Planned the experiment with supervisors, performed all experimental and practical work, evaluated and analysed the data and wrote the manuscript in collaboration with the co-authors.

Abbreviations

ANOVA	Analysis of Variance
ASPP	Amount and Size Distribution of Polymeric Proteins
CDMT	Cultivar determined plant maturation time
DAA	Days after anthesis
E	Environment
e	extractable
FAO	Food and Agriculture Organization
G	Genetic
GMP	Grain maturation period
GPC	Grain protein concentration
GY	Grain yield
HMW-GS	High Molecular Weight Glutenin Subunits
h	hours
kDa	Kilodalton
LMW-GS	Low Molecular Weight Glutenin Subunits
LTP	Lipid transfer protein
Mon/pol	Ratio of monomers to polymers
MP	Monomeric proteins
N	Nitrogen
NA	Nitrogen amount
PCA	Principal Component Analysis
PDI	Protein disulphide isomerase
PP	Polymeric proteins
QTLs	Quantitative trait loci
RA	Relative addition rate of nitrogen
S	Sulphur
SAS	Statistical analysis system
SDS	Sodium Dodecyl Sulphate
SCB	Statistiska centralbyrån
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SE-HPLC	Size Exclusion High Performance Liquid Chromatography

SMP	Small monomeric proteins
TCA	Trichloroacetic acid
TOTE	Total SDS extractable proteins
TOTU	Total SDS un-extractable proteins
TSMP	Total small monomeric proteins (SDS extracted and un-extracted)
u	Un-extractable
%LargeUPP	Percentage of large un-extractable polymers into total large polymers
%LUMP	Percentage of large un-extractable monomers into total large monomers
%SUMP	Percentage of small un-extractable monomers into total small monomers
%UPP	Percentage of un-extractable polymers into total polymers

1 Background and introduction

In agriculture, cereals (especially wheat and barley) are considered important not only in terms of adaptability, production and consumption, but also in providing food and energy for humans and animals. It has been estimated that global cereal consumption directly provides about 50% of the necessary protein and energy within the human diet, with cereals providing an additional 25% of protein and energy via livestock intermediaries (Shewry, 2007). Between now and the year 2050, the human population is expected to increase to 9.5 billion and for this reason it will be necessary to increase agricultural and especially cereal production 1.7 fold (Hirel & Lea, 2011). Therefore, the challenge for the coming decades will be to develop cereal crops with high yields, but also with desirable quality and simultaneously with a focus on sustainability of the environment (Dyson, 1999). There are a number of factors *e.g.* genetic (G), environmental (E), agronomic *etc.* that affect the yield and quality of cereals. Thus, it is important to understand the background of differences caused by G and E factors, individually and in combination, on the yield and quality of wheat and barley in order to achieve profitable yields and quality for desirable end-uses.

1.1 Importance of wheat and barley

Cereals are the most important crops cultivated worldwide, with an annual production of 2229 million tons (FAO, 2010) and they account for the majority of products and end-products used in human diet. Wheat is one of the major world cereal crops, with a total global production of about 651 million tons in 2010 (FAO, 2010). Barley is ranked fourth in cereals grown worldwide reaching a cultivated area of 54 million hectares and with a total production of 152 million tons in 2010 (FAO, 2010). In Sweden too, wheat is one of the major cereal crops with an annual production of 2.2 million tons (SCB, 2011).

In terms of production, barley is the second largest cereal crop in Sweden after wheat, with an annual production of 1.4 million tons (SCB, 2011).

1.1.1 History and domestication of wheat and barley

The domestication of cereals started about 12,000 years ago when humans made the shift from hunting to plant cultivation for their survival during the Neolithic revolution (Shewry, 2009). The first agriculture practices have been attributed to Fertile Crescent - a region that covers modern day Israel, Jordan, Lebanon and western Syria, into south-east Turkey and along the Tigris and Euphrates rivers, into Iraq and the western flanks of Iran (Dubcovsky & Dvorak, 2007; Heun *et al.*, 1997).

The tribe Triticeae, which belongs to the grass family Gramineae (Kellogg, 2001), includes several of the world's most important cereal crops, such as wheat, barley, triticale and rye (Kawahara, 2009). Most commonly five genera *i.e. Aegilops*, *Elymus*, *Triticum*, *Secale* and *Hordeum* are included in the tribe Triticeae, irrespective of the classification system used. All 23 species of wheat belong to the genus *Triticum* and all 31 species of barley belong to the genus *Hordeum*, both genera form polyploidy series with a basic chromosome number x = 7 (Baden & Bothmer, 1994).

The wild species of *Triticum* are diploid i.e. *T. monococcum* with genome AA, *T. tauschii* (DD) and *T. speltoides* (SS) and their chromosome number is 2n = 2x = 14. The tetraploid species *i.e. T. turgidum* (AABB) have chromosome number 2n = 4x = 28 and the modern bread wheat *T. aestivum* (AABBDD) is hexaploid with chromosome number 2n = 6x = 42 (Zohary & Hopf, 1993). Due to the polyploid nature of wheat, there is great potential for genetic variation and about 17, 000 wheat cultivars had been produced in the beginning of 1970s (Feldman, 1976).

The cultivated barley, *Hordeum vulgare* L. ssp. *vulgare*, and its wild progenitor *H. vulgare* L.ssp. *spontaneum* are diploid species, with 2n=2x=14 chromosomes. Other *Hordeum* species are diploid, tetraploid (2n=4x=28) or hexaploid (2n=6x=42). Cultivated barley is divided into three subgroups, six-row (*Hordeum vulgare*), two-row (*H. distichum*) and intermediate (*H. irregulare*), with both spring- and autumn-sown types. Barley is also classified as hulled or hulless by presence or absence of hull tightly adhering to the grain (Baik & Ullrich, 2008).

1.1.2 Uses of wheat and barley

Wheat, due to its unique baking qualities, taste and long shelf-life compared with other cereals such as barley, is the most preferred cereal crop for bread, noodles, pasta, *etc.* (Dewettinck *et al.*, 2008). About 100 million tons, of the

wheat produced annually, are traded internationally while the remainder is utilised within the country of origin (Shewry et al., 2012). In Sweden, a major proportion of the wheat produced is used for animal feed, whilst the rest is used either for bio-fuel production or for direct human consumption as bread, breakfast cereal, pasta, table wheat, *etc.* (Jordbruksverket, 2011).

About two-thirds of the barley grown worldwide is used for animal feed, one-third for malting purposes and around 2% for human food (Baik & Ullrich, 2008; Newman & Newman, 2006). The barley grain components with unique nutritional and industrial properties make barley suitable for malting and beer production (Xue *et al.*, 2008). According to end-use, barley is categorised as malting or feed type (Baik & Ullrich, 2008). Both two-row and six-row barley are considered sutiable for malting, but the best malt quality for beer is produced from two-row spring-sown barley varieties. The Swedish malting barley crop amounted 435600 tons in 2011 (www.vikingmalt.com).

1.2 Grain composition

1.2.1 Wheat

The wheat grain consists of starch (70-75%), water (14%), proteins (8-20%), non-starch polysaccharides (2-3%), lipids (2%), minerals (1.6%) and other smaller constituents such as antioxidants (Goesaert *et al.*, 2005).

Wheat proteins

On the basis of sequential extractions in a series of solvents, the proteins in wheat are classified into four types, namely albumins (water-soluble), globulins (salt-soluble), prolamins (aqueous alcohol-soluble) and glutelins (alkali-soluble) (Osborne, 1924). Wheat grain proteins are also classified into structural/metabolic (non-gluten) and storage (gluten) proteins (Shewry, 2003) (Figure 1).

The non-storage proteins albumins and globulins constitute 15-20% of the total wheat grain proteins and are responsible for enzymatic activity and starch breakdown (Singh & Skerritt, 2001; Pence *et al.*, 1954).

The storage proteins (gluten) constitute up to 80-85% of the total wheat grain proteins (Shewry *et al.*, 1995). Wheat storage proteins are synthesised as polypeptides on the polyribosomes, which are attached to the endoplasmic reticulum and are located in the starchy endosperm of the developing wheat kernel. After synthesis, the polypeptides are translocated to the lumen cavity, where disulphide bonding and protein folding occur (Shewry *et al.*, 2002). For deposition and transportation of proteins, two pathways have been suggested: 1) via the Golgi apparatus to the vacuole and then to the final deposition place;

or 2) directly to the protein bodies (Shewry, 1999; Parker, 1980). Gluten plays an important role in determining the bread-making quality of wheat due to its visco-elastic properties (MacRitchie, 1984). The main components of the gluten proteins are gliadins and glutenins in wheat (Shewry & Tatham, 1990).

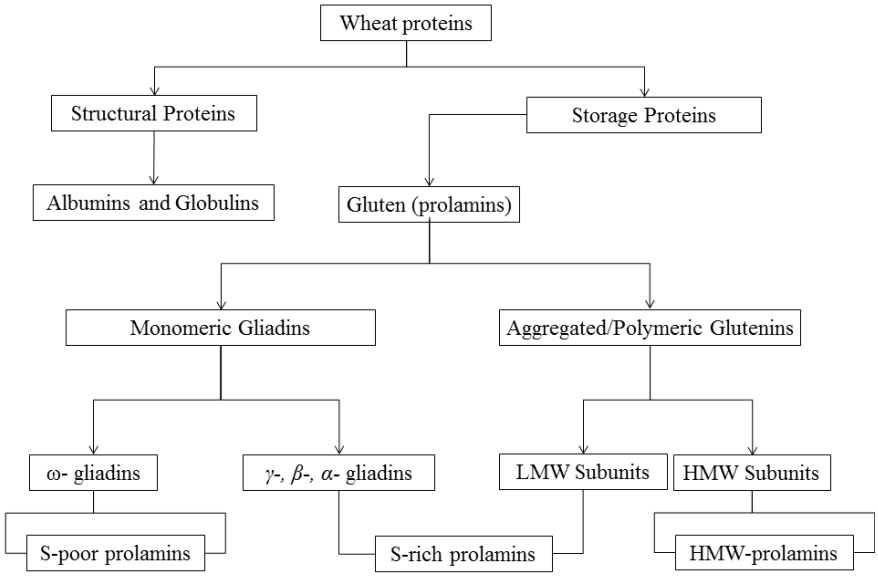

Figure 1. Wheat protein classification (adapted from Shewry & Tatham, 1990. HMW=High molecular weight, LMW= Low molecular weight, S= sulphur

Gliadins constitute 40-45% of the total wheat grain proteins and are monomeric in nature. On the basis of molecular mobility at low pH, gliadins are classified into four groups, α, β, γ and ω. The molecular weight of gliadins ranges between 30 and 80 kDa (Shewry *et al.*, 1986). According to sequences, composition of amino acids and molecular weights, gliadins are further grouped into α/β-, γ-, ω1-, ω2- and ω5-gliadins (Wieser, 2007). Gliadins are responsible for giving the viscosity to the wheat flour and are less elastic than glutenins (Wieser, 2007).

Glutenins constitute around 40-45% of the total wheat grain proteins and are extractable in dilute acetic acid. Glutenins are polymeric in nature (Field *et al.*, 1983). In wheat, two classes of glutenin subunits, high molecular weight-glutenin subunits (HMW-GS) and low molecular weight-glutenin subunits (LMW-GS) are present. On the reduction of disulphide bonds, in glutenins, by using reducing agents, HMW-GS and LMW-GS are released (Wang *et al.*, 2006). HMW-GS constitute around 10% and LMW-GS constitute 40% of the

gluten proteins (glutenins and gliadins; Payne, 1986). HMW-GS and LMW-GS have been found to correlate with bread-making quality and gluten strength (Gupta *et al.*, 1993).

1.2.2 Barley

The barley grain contains starch (63-65%), water (10-15%), proteins (9-13%), non-starchy polysaccharides (9-11%), lipids (1-2%), minerals (2%) and other smaller constituents (Chibbar *et al.*, 2004; MacGregor & Fincher, 1993).

Barley proteins

Similarly as in wheat, the proteins in barley are divided into four groups on the basis of their extractability, *i.e.* albumins (water-soluble), globulins (salt-soluble), hordeins or prolamins (alcohol-soluble), and glutelins (alkali-soluble; Osborne, 1924; Figure 2).

The content of non-storage proteins, *i.e.* albumins and globulins, in the barley grain is somewhat higher, 11% and 15%, respectively, than that in wheat. However, albumins and globulins have the same functions in barley as in wheat (Steiner *et al.*, 2011; Briggs & Hough, 1981). On a functional basis, some important albumins in barley are z-proteins and lipid transfer proteins (LTP) (Steiner *et al.*, 2011). These z-proteins and LTP play an important role in beer foam formation and stability (Evans *et al.*, 1999).

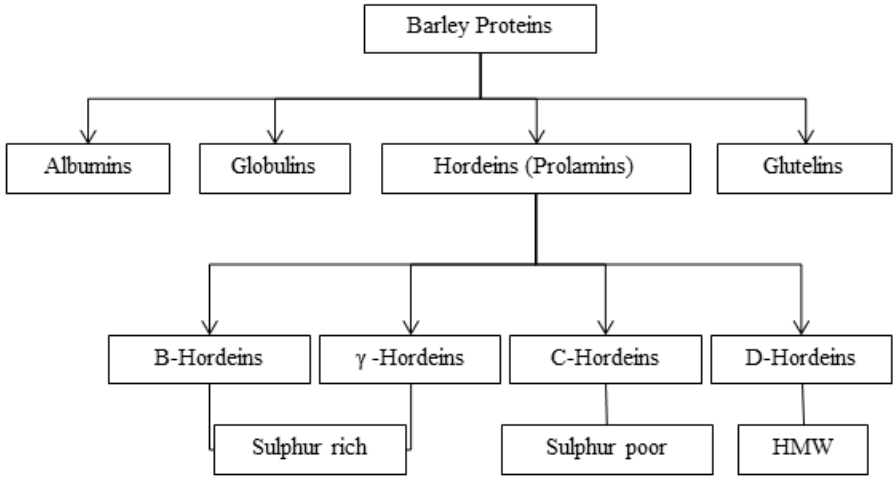

Figure 2. Barley protein classification. Adapted from Steiner *et al.* (2011). HMW= High molecular weight.

Barley glutelins constitute 30% of the total barley protein (Shewry *et al.*, 1988). The content of prolamins in barley (*i.e.* hordeins) constitutes 37-44% of the total barley grain proteins (Osman *et al.*, 2002; Shewry *et al.*, 1995; Rahman *et al.*, 1982). Similarly to the gluten proteins, the barley storage proteins, the glutelins and prolamins, are also synthesised in the rough endoplasmic reticulum during mid-to-late grain filling (Rahman *et al.*, 1982) and accumulate in the protein bodies inside the endomembrane system (Matthews & Miflin, 1980). Hordeins are divided into different fractions according to their size and amino acid composition. The A-hordeins (15-25 kDa) are not considered as true storage proteins because of the presence of protease inhibitors and α-amylases (Tatham & Shewry, 1995). The B-hordeins (molecular weight 35-46 kDa), the γ-hordeins (molecular weight <20 kDa), the C-hordeins (molecular weight 55-75 kDa) and the D-hordeins (molecular weight >100 kDa) account for 70-90%, 1-2%, 10-30% and 2-4% of the total hordein fraction, respectively (Shewry *et al.*, 1985). The B-hordein fraction can be further subdivided into B1, B2 and B3 (Skerritt & Janes, 1992). The C-hordeins and some B-hordeins appear as monomers; however, most B-hordeins and D-hordeins are linked with each other by inter-chain disulphide bonds and appear as polymers (Celus *et al.*, 2006)

1.3 Factors affecting grain yield, protein concentration and composition in wheat and barley grain

Higher grain yield (GY) is often associated with lower grain protein concentration (GPC) in wheat and barley (Simmonds, 1995). However, low GPC at the expense of high GY is undesirable. One of the most important breeding goals nowadays is to increase GYs without adversely affecting GPC by selection and breeding of genotypes from old and new germplasm (Hirel & Lea, 2011). GPC can also be increased by greater mobilisation of N from vegetative parts, including roots, to reproductive parts. Thus, it is important to understand the relationship between dry weight and N concentration in plant parts (including roots) and GPC and GY.

GPC and grain protein composition are considered the most essential determinants of bread-making quality in wheat (Finney & Barmore, 1948). The GPC of wheat is governed not only by the G background but also by E factors *e.g.* nitrogen (N) fertiliser, precipitation and temperature conditions during growth and development (Zhang *et al.*, 2001; Johansson *et al.* 2001; McDonald, 1992). In malting barley, the GPC should be lower than 11.5% in order to achieve the desirable malting quality (Palmer, 2000; Bertholdsson, 1999), while in bread wheat a high GPC is often desired (Johansson &

Svensson, 1998; Finney & Barmore, 1948). It is quite difficult to keep the GPC in an acceptable range as GPC is influenced by cultivation and agronomic practices, as well as E factors such as N availability *etc.* (Zhang *et al.*, 2001; Bertholdsson & Stoy 1995; Eagles *et al.* 1995).

Since GPC varies widely as an effect of different E and G factors, the importance of grain protein composition cannot be neglected in relation to end-use quality purposes. Specific protein composition in wheat and barley is determined by G factors, but the amount of different protein groups, the amount and size distribution of polymeric proteins (ASPP) and monomeric proteins are influenced by E factors *i.e.* N fertiliser and temperature, as well as G factors (Johansson *et al.*, 2001, 2005, 2008). In barley, protein composition has also been found to be influenced by different G and E factors, as is malting quality (Wang *et al.*, 2007).

Growth and development stages of the wheat and barley crop before and after flowering may play a significant role in GPC and protein composition in the grain. In earlier studies the rate and duration of grain maturation period (GMP) have been found to affect the GPC, protein accumulation and composition in wheat and barley (Johansson *et al.*, 2005, 2008; Dreccer *et al.*, 1997). However, few studies to date have focused on the connections between maturation times (*i.e.* time to anthesis and GMP) and protein parameters in wheat and barley. Plant maturation times are influenced not only by G factors but also to a large extent by E factors (*e.g.* Wang *et al.*, 2007; Conry, 1997). In wheat and barley, the relationship between maturation time and GPC and protein composition has not been investigated in any great depth. Many investigations have been carried out on the individual influence of G and E factors on GY, GPC, protein accumulation and protein composition in the grains of wheat and barley (Wang *et al.*, 2007; Johansson *et al.*, 2005, 2008). However, few studies have investigated the interactive impact of G and E factors on GY, GPC, protein accumulation and composition in wheat and barley grains. Moreover, very limited investigations have been carried out on the influence of G and E factors on the breakdown of proteins during the malting process in barley.

2 Objectives

The main aim of this doctoral thesis was to identify the options that can be used to govern GPC and protein composition in wheat and barley grain by the use of G and E factors. To reach this main aim, focus of this thesis work has been to study how individual and interactive effects of G and E factors determine GPC and protein composition in wheat and barley. Further, the influence of G and E factors on build-up and breakdown of proteins during cultivation and at malting was evaluated. Importance and interactions of various G and E factors for build-up of proteins, for final GPC and protein composition at maturity and during breakdown of proteins at malting have also been investigated.

Specific objectives were:

- To evaluate genotypic variation in dry weight and N concentration in wheat plant parts (especially root dry weight and root N concentration and their relationships with GY and GPC.
- To evaluate the individual and interactive effect of different locations, optimised nutrient composition, G, agronomic and E factors on the growth, GY, GPC, protein accumulation and composition of wheat and spring malting barley.
- To evaluate the individual and interactive influence of G, agronomic and E factors in governing the maturation times of wheat and barley plants.
- To study the impact of G and E factors on the degradation of polymeric proteins into peptides and amino acids in malted grains of barley during the malting process.
- To study how interactions between G and E factors affect the accumulation and composition of proteins in wheat and barley grains.

3 Materials and Methods

3.1 Plant material (Papers I-VII)

The investigations were carried out with wheat and barley cultivars with different genetic backgrounds, origins (Finland, Norway, Pakistan, and Sweden) and release years (Paper I-VII). Detailed descriptions of the wheat and barley cultivars can be found in the individual papers.

3.2 Cultivation conditions (Papers I-VII)

The wheat and barley plants in the experiments were grown in either soil or hydroponic cultivations to full maturity in controlled and daylight climate chambers. In several of the soil-based cultivations, the wheat and barley plants were planted in large boxes to resemble field conditions. Moreover, N was broadcast at different growth stages of the wheat and barley plants. In Paper II, the soil was dried before sowing to minimise the influence of microbial activity on soil chemical profile. The hydroponic cultivations were performed according to Andersson & Holm (2011), Johansson *et al.* (1994) and Mattsson *et al.* (1991). In the hydroponic systems, the N supply was controlled by daily dosage and the nitrogen amount (NA) was set according to the equation:

$$NA_t = NA_{t-1}\ eRA$$

where NA_t is the NA of the plant at day t and NA_{t-1} the NA at day t-1 (with the daily N dose calculated from $NA_t - NA_{t-1}$) and RA is the relative addition rate of N, *i.e.* the rate at which N is supplied to the plants (NA added plant^{-1} day^{-1}). RA was altered during the phenological development of the plants. When the RA is kept at a growth-limiting level, the N added daily is taken up within 24 h and is equal to the relative increase in plant N amount (Oscarson, 1996). The N supply was thus intended to mimic the N uptake in the field-grown wheat.

3.3 Nitrogen/protein concentration (Papers I, II, IV, VI)

For measuring N concentration in different plant parts, these were dried, weighed, ground and analysed for N using the Dumas method through volatilisation of N in a Carlo Erba N analyser. To calculate GPC in the grains, the N concentration was multiplied by a conversion factor of 6.25 according to Mosse (1990).

3.4 Protein composition (Papers II, III, IV, V, VI, VII)

The protein composition of wheat and barley grains was evaluated as ASPP according to Gupta *et al.* (1993) by applying size exclusion-high performance liquid chromatography (SE-HPLC) with a two-step procedure according to Johansson *et al.* (2005). The first step involves extraction of SDS-extractable (e) proteins, while the second step involves extraction of SDS-unextractable (u) proteins by sonication. Polymeric proteins (PP) and monomeric proteins (MP) were extracted with dilute SDS phosphate buffer. A detailed description of the HPLC system and the phase used *etc.* can be found in Papers II-VII.

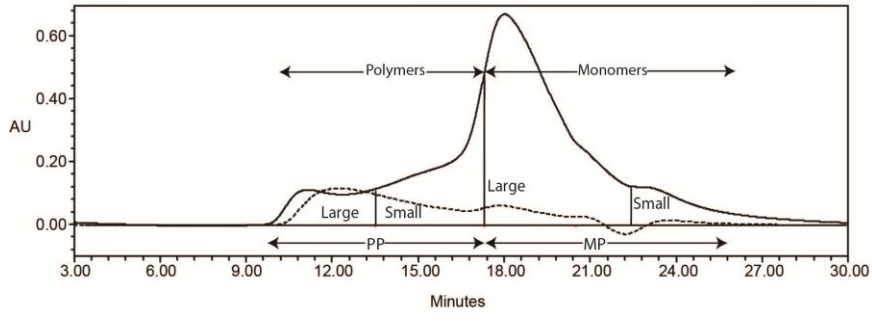

Figure 3. Example of SE-HPLC chromatogram of SDS-extractable proteins (—) and SDS-unextractable proteins (---). The chromatogram is divided into two parts, comprising polymeric proteins (PP) and monomeric proteins (MP), respectively. AU=Absorbance units of UV detector at 210 nm.

Moreover, different protein fractions were calculated according to area under the chromatogram as follows:

- Total SDS-extractable proteins (TOTE) = ePP + eMP
- Total SDS-unextractable proteins (TOTU) = uPP + uMP
- Total small monomeric proteins (TSMP) = eMP (small) + uMP (small)
- Percentage of large unextractable polymeric protein in total large polymeric proteins (%Large UPP) = uLPP/(uLPP + eLPP) × 100
- Percentage of total unextractable polymeric protein in total polymeric proteins (%UPP) = uPP /(ePP + uPP) × 100
-

- Percentage of large unextractable monomers in total large monomers (%LUMP) = uLMP/(uLMP + eLMP) ×100
- Percentage of small unextractable monomeric protein in total small monomeric protein (%SUMP) = uSMP/(uSMP + eSMP) ×100
- Mon/pol (ratio of monomers to polymers) [(SDS-extractable MP+SDS-unextractable MP)/(SDS-extractable PP+SDS-unextractable PP)]

3.5 Specific protein composition (Paper V)

The specific grain protein composition (HMW-GS) in wheat grains was determined with SDS-PAGE according to the methods and scoring described by Payne *et al.* (1983). Coomassie Brilliant Blue solution with 10% ethanol and 8% trichloroacetic (TCA) acid was used to stain the gels while to destain the gels, a water solution with 4% TCA was used for 24 h to obtain a clear banding pattern according to Johansson *et al.* (1993).

3.6 Malting and endosperm modification (Papers II, VI)

Malting was carried out in the micro-malting plant at SLU, Alnarp, Sweden (Danbrew Consult Ltd, Copenhagen V, Denmark) according to Henry & McLean (1984) in three steps *i.e.* steeping, germination and kilning. In order to check how much protein had been modified in the endosperm during malting, endosperm modification was determined according to Henry (1989). This involved embedding and sectioning the grain, staining with Calcofluor and Fast Green and observation under fluorescent light.

3.7 Statistical analyses (Papers I-VII)

MS Excel and the statistical analysis system (SAS) (SAS, 2004) together with Minitab were used for figures and data analysis, respectively. Data evaluation was done by Spearman rank correlation analysis, analysis of variance (ANNOVA), principal component analysis (PCA) and regression analysis using SAS and Minitab statistical software (Multivariate, v. 16, Minitab Inc.).

4 Results and discussion

4.1 Factors influencing grain protein concentration and composition

GPC and protein composition are well known to be governed by G and E factors in both wheat and barley (Zhang *et al*., 2001; Johansson & Svensson, 1998; Eagles *et al*., 1995; Kramer, 1979). The contribution of various G and E factors to quality are ambiguous in different studies. Studies on G influences (on GPC and protein composition) not only include everything from specific genes to quantitative trait loci (QTLs), but also cultivar variations and plant physiology-related variations (Uauy *et al*., 2006; Johansson *et al*., 2005, 2008; Blanco *et al*., 2002; Payne *et al*., 1983). As regards E influences on GPC and protein composition, several studies refer to temperature, climate and agro-ecological conditions, although some studies also focus on influences of fertiliser (especially N), soil properties, year or location effects and others consider agronomy-based variations (Vázquez *et al*., 2012; Oelofse *et al*., 2010; Wang *et al*., 2007; Johansson *et al*., 2005, 2008; Dupont & Altenbach, 2003).

It is well established that GPC in wheat and barley is under the control of G factors, with a number of major genes and QTLs seeming to be involved (Bogard *et al*., 2011; Ullrich, 2010; Uauy *et al*., 2006). The G influence on GPC might be directly genetically governed, meaning that one gene in a certain cultivar results in a higher GPC than in another cultivar due to the presence of that certain gene (Uauy *et al*., 2006; Joppa & Cantrell, 1990). However, in most cases, genes involved in the amount of GPC are related to other characters of the plant (*e.g.* N assimilation and transportation, *etc*.), which thereafter influence the GPC (Habash *et al*., 2007; Levy & Feldman, 1987; Cox *et al*., 1986). In this thesis, no studies were undertaken to evaluate the influences of certain genes on GPC. Instead, the emphasis was on evaluating

the background of GPC by the use of various genotypes and the variation in certain characters that might be of relevance for determination of GPC. The results presented in the thesis show a large variation in GPC among genotypes (Figure 4; Paper I). However, no plant physiological relationships for this variation were found other than a negative correlation with grain weight (Paper I). Furthermore, plant maturation times were found to correlate with GPC (Papers IV, V, VI and VII). In both wheat and spring malting barley, early maturation times resulted in high GPC or TOTE (Papers IV, V, VI and VII). The amounts of TOTE and GPC were found to be strongly correlated in this thesis (Papers II, IV and VII) and also in previous investigations (Godfrey *et al*., 2010; Johansson *et al*., 2005, 2008).

A number of E factors (starter fertiliser, N amount and timing, pre- and post-temperature and soil) were evaluated in this thesis for their influences on GPC. Fertiliser treatments, both in terms of starter fertiliser and N amount in total, were found to influence GPC to a small degree or not at all (Papers II, VI). However, N timing in terms of late N application was found to increase GPC in wheat and barley (Papers IV, V, VI and VII), as also reported previously in a number of studies (Johansson *et al*., 2001, 2003, 2005; Wieser & Seilmeier, 1998). Late N application leads to increased and late transport of N to the grains and therefore directly influences the N concentration in the grain (Ferrise *et al*., 2010; Bancal *et al*., 2008). The choice of soil directly influenced the GPC in the grains, which could probably have been expected due to the variations in N content and mineralisation from various soils (Paper II). Temperature before anthesis was found to influence GPC substantially, while temperature after anthesis was of less importance (Paper IV). An increased temperature before anthesis shortens the plant maturation time until anthesis and thereby leads to less assimilation of carbohydrates in the plant (Bertholdsson, 1999). A lower accumulation of assimilates thereafter leads to a lower starch accumulation in the grain and thereby to higher GPC (Savin & Nicolas, 1996).

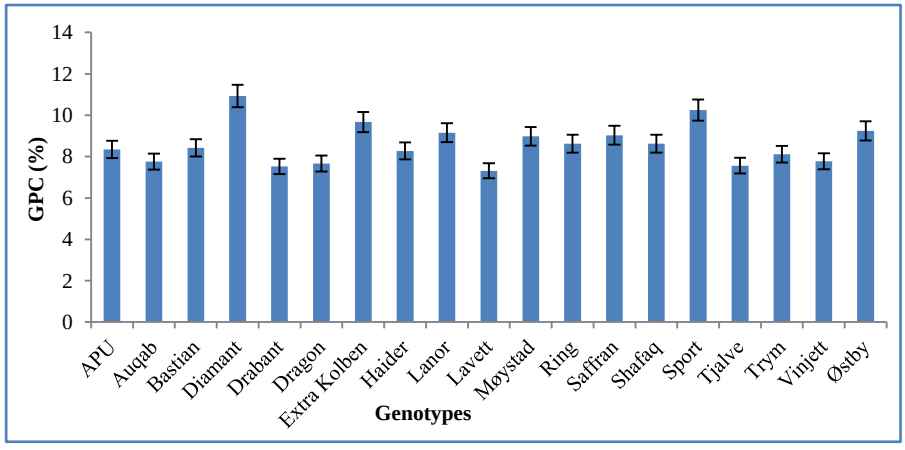

Figure 4. Mean grain protein concentration (GPC, %) of 19 wheat genotypes with different origins and release years (Paper I).

Grain protein composition was determined in this thesis as a number of protein factors, although most emphasis was placed on %UPP (Papers II, IV, V and VI), as this parameter is known to correlate with gluten strength and has been widely studied (Johansson, 2002; Marchylo *et al*., 1989). Percentage of UPP is known to be G-determined by its relationship to specific protein composition (Gupta & MacRitchie, 1994; Shewry *et al*., 1992), but additional G influences are also evident due to its variation between genotypes with the same specific protein composition (Johansson *et al*., 2002, 2003). In this thesis, the influence of cultivar-determined plant maturation time (CDMT) on %UPP is clearly demonstrated for the first time (Papers V, VI and VII). Late maturation time of a cultivar was correlated with an increased %UPP (Paper VII) and maturation time explained variation in %UPP to a higher extent than specific protein composition. The reason for the relationships between CDMT and %UPP might be due to the negative correlation between TOTE (GPC) and %UPP (Table 1; Papers II, IV, V and VI). An increased GPC is often related to a higher increase in ethanol-soluble proteins (monomeric proteins) than non-ethanol soluble proteins (polymeric proteins) (Johansson *et al*., 2003; Wieser & Seilmeier, 1998).

Among the E factors evaluated here (see above), it was mainly N application timings and temperature during GMP that influenced %UPP (Papers V and VI). Early N application led to increased %UPP (Papers V and VI), which could be explained by the fact that early N availability for the plant prolongs its maturation time. Thus, a similar explanation could be applicable for early N application as for CDMT. The fact that increased temperature

during GMP resulted in increased %UPP can be due to a number of reasons. First, the enzymatic activity, including PDI (protein disulphide isomerase), involved in protein polymerisation might be altered, since enzymes are normally temperature-dependent (Every *et al*., 2003; Hurkman *et al*., 2003). Secondly, water content in the grain is well known to influence %UPP (Naeem *et al*., 2012; Johansson *et al*., 2008) and the hydrogen bonds between proteins are known to be influenced by the presence of water (Stryer, 1981). Thirdly, there is some influence of the temperature during GMP on GPC (Papers IV, V and VI and VII), and changes in GPC also create changes in %UPP, as described above.

Table 1. *Spearman rank correlation between GPC and protein composition in spring malting barley grains grown at two different locations with and without starter fertiliser application (Paper II)*

	TOTE	%UPP	%LargeUPP	Mon/pol
GPC	0.59	-0.52	-0.59	0.60
P value	*0.0024*	*0.0096*	*0.0025*	*0.0019*

*, **, ***= Significant at P<0.05, 0.01, 0.005

4.2 Relationships between grain yield, protein concentration and composition

The negative relationship between GY and GPC is well known in cereals (Simmonds, 1995). A strong negative correlation was observed in GY and GPC in our investigations with wheat and barley (Papers I, II and VI). GY is mainly dependent on the carbohydrate deposition in the grain, while N deposition is mainly responsible for GPC (Jenner *et al*., 1991). Although starch and protein synthesis are seen as independent events (Jenner *et al*., 1991), a negative trend between GY and GPC describes the inter-relationship between carbon and N metabolism (Acreche & Slafer, 2009). In this thesis, GY and GPC were evaluated in a number of genotypes. Genotypes having a relatively high GY, and combined with either low or high GPC, were found (Figure 4; Paper I). Genotypes with a desired combination of GY and GPC are of relevance both in bread wheat and malting barley, since both require high GY but bread wheat requires high GPC and malting barley requires low GPC.

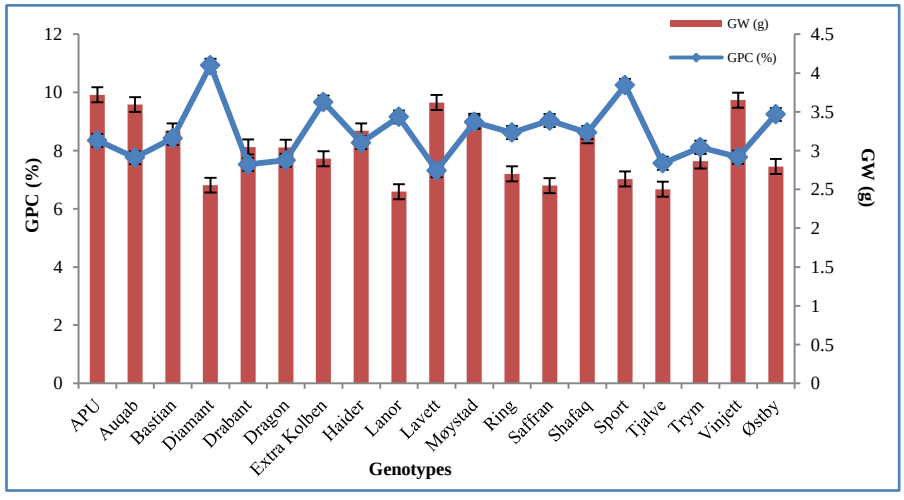

Figure 5. Mean values of grain protein concentration (%) and grain weight (GW) in 19 wheat genotypes with different origins and release years (Paper I).

The relationship between GY and grain protein composition in wheat and barley has not previously been investigated. In spring malting barley (Paper II and IV), GY was found to be significantly and negatively correlated with SDS-extractable proteins. However, a positive correlation was observed between GY and SDS-unextractable polymeric proteins (Table 2). Possible relationships between GY and %UPP can most likely be explained by the fact that there is a relationship between GY and GPC, and between GPC and %UPP. In governing GPC and protein composition, GY is of little relevance.

Table 2. *Spearman rank correlation between grain yield, grain protein concentration and grain protein composition in spring malting barley grains grown at two different locations with and without starter fertiliser application (Paper II)*

	GPC	TOTE	TSMP	TOTU	%UPP	%LargeUPP	Mon/pol
Grain yield	-0.95	-0.65	-0.61	-0.10	0.62	0.71	-0.59
P value	<.0001	0.0005	0.0013	0.6251	0.0012	<.0001	0.0021

*, **, ***= Significant at P<0.05, 0.01, 0.005

4.3 Build-up of proteins and relationship to protein concentration and composition in mature grains

From previous investigations it is well known that variations in E factors such as N and temperature are responsible for the build-up of ASPP and monomeric proteins during the GMP (Johansson *et al*., 2005, 2008). The build-up and polymerisation of the gluten proteins have been reported to be a predetermined event (Johansson *et al*., 2005, 2008; Stone & Nicolas, 1996). Temperature-sums have been cited as the main factor in the onset of the build-up of polymers (Triboi *et al*., 2003; Stone and Nicolas, 1996). The results presented in this thesis confirm the predetermined nature of the grain protein polymers in wheat (Papers III and V). Furthermore, both the GPC and the protein composition in terms of *e.g*. %UPP were found to be largely dependent on E factors during plant maturation (Papers III and V).

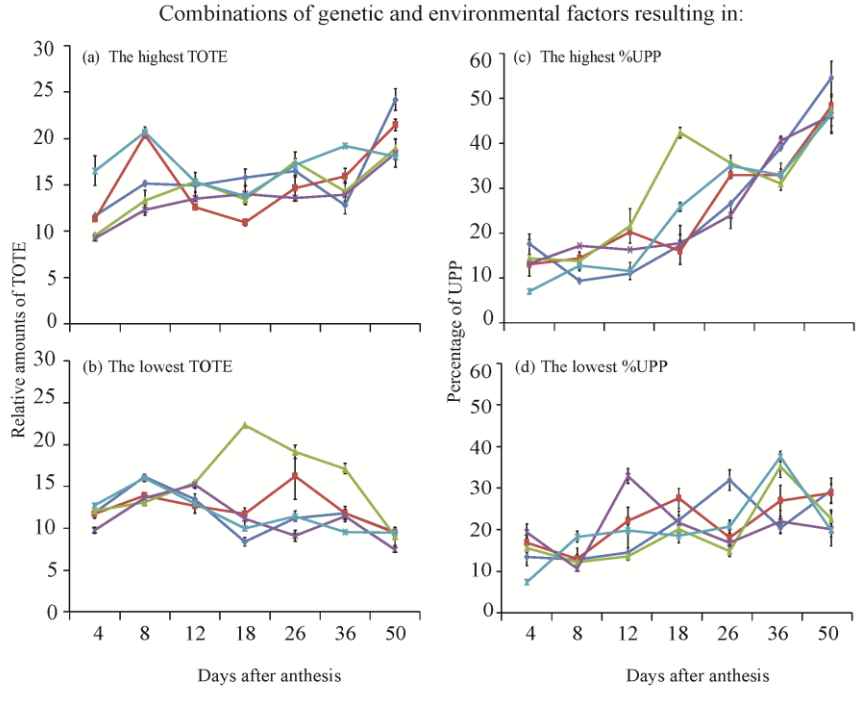

Figure 6. Build-up of relative amounts of total SDS-extractable proteins (TOTE) and SDS-unextractable polymeric protein in total un-extractable polymeric protein (%UPP) from 4 to 50 days after anthesis for selected combinations of G and E factors resulting in the highest versus the lowest amounts of TOTE (6a and 6b) and %UPP (6c and 6d), respectively.

However, by selecting G and E factors that resulted in high and low TOTE and %UPP at maturity, this thesis showed for the first time that it is possible to differentiate a starting time for the plant when the level of GPC and polymerisation of the proteins are settled (Figure 6a-6d; Papers III and V). Thus, a high or low level of TOTE and %UPP at maturity was settled already at 12 days after anthesis (DAA; Papers III and V). Before 12 DAA there was no clear variation in the build-up of TOTE and %UPP, although the plants were differently E-treated during the whole GMP (Papers III and V). However, from 12 DAA the variation in the speed of build-up of TOTE and %UPP started and continued throughout the GMP. For the combinations of G and E factors resulting in high or low TOTE and high %UPP, at maturity, the increase was steady from 12 DAA. For the combinations of G and E factors resulting in low %UPP at maturity, no increase in %UPP could be seen during the whole GMP (Papers III and V).

4.4 Interactions of various factors in governing protein concentration and composition

In most previous studies, single G or E factors have been evaluated for their impact on GPC and protein composition (Johansson & Svensson, 1998, 1999; Peterson *et al*., 1992). The work in this thesis comprises by far the most thorough examination to date of how various G and E factors interact in determining GPC and protein composition in wheat and barley. One conclusion that can be drawn from the investigations within this thesis is that the GPC and protein composition in wheat and barley are very similar and that the two species react similarly to G and E factors and their interactions (Papers II-VII).

Generally, a combination of G and E factors such as CDMT, variation in temperature before and after anthesis, N amount and timing, soil and starter fertiliser showed a larger impact on GPC and protein composition than any of these individual factors alone (Papers II, IV, V, VI and VII). More specifically, application of starter fertiliser using soil originating from Laxmans Åkarp resulted in higher GPC in spring malting barley than when a combination of starter fertiliser and/or soils of other origin was used (Paper II).

High amounts of TOTE in wheat were found to be correlated with a combination of early maturing cultivars, late nitrogen application and low temperature during GMP (Paper V). Furthermore, a combination of late sowing, 50/50% N at sowing/flowering and high temperature during GMP resulted in high TOTE in mature barley grains (Paper VI). In addition, high pre-anthesis temperature and short time to anthesis resulted in high amounts of TOTE in barley grains (Paper IV). Thus, all factors resulting in a reduced plant

maturation time before anthesis contributed to a higher TOTE and probably to a lower GY (although the latter was not investigated in this thesis).

As to protein composition, combinations of cultivars with relatively late maturation time together with early nitrogen application resulted in high %UPP (Paper V). Furthermore, %UPP in mature barley grains was found to be governed by a combination of sowing times, N application rate and timings and temperature during the GMP in barley grains (Paper VI). Low %UPP was obtained by a combination of late sowing time, 50% N at sowing and high temperature during the GMP (Paper VI). It was also found that high post-anthesis temperature and longer CDMT were associated with high %UPP (Paper IV).

4.5 Breakdown of proteins and relationship to protein concentration and composition in mature grains

The breakdown of proteins in cereal grains during germination of the seed is important to produce the coming generation of plants (Yang *et al*., 2007; Müntz *et al*., 2001). Thus without the breakdown of proteins, there will be no germination to produce the next generation of plants and the genus/cultivar will cease. However, if the breakdown of proteins is too rapid, this is often connected with the rapid breakdown of starch, and thus problems of pre-harvest sprouting, low falling number and poor quality will arise (Mares & Mrva, 2008). Furthermore, during the malting process of barley, the breakdown of grain proteins into peptides and amino acids by a range of proteolytic enzymes is considered to be of critical importance in determining malt quality (Jones, 2005; Baxter, 1981). The breakdown of proteins during malting provides sufficient nutrients for yeasts to grow rapidly and to metabolise sugars into alcohol (Steiner *et al*., 2011; Celus *et al*., 2006). However, complete degradation of all barley proteins during malting and beer production is not desirable. Too low protein content in the beer (the main product made from malt) may result in a product that has insufficient foaming ability, mouth feel and other required characteristics (Steiner *et al*., 2011; Celus *et al*., 2006; Jones, 2005). The desirable proteins in the beer are mainly some specific albumins, *e.g.* z-proteins and LTP (Steiner *et al*., 2011; Silva *et al*., 2008; Evans *et al*., 1999).

This thesis shows for the first time that certain G- and E-related influences during plant maturation affect the breakdown rates of various types of proteins at malting (Papers II and VI). Soil from specific locations combined with/without starter fertiliser (Paper II), variations in CDMT (Paper VI) and a combination of sowing time and N application timing (Paper VI) were found to

influence the breakdown rates of the proteins. The breakdown of proteins during malting was high in grains grown in soil from Laxmans Åkarp combined with no starter fertiliser and in soil from Lunnarp combined with starter fertiliser (Paper II). Furthermore, extensive degradation of TOTE was associated with late sowing, 50/50% N at sowing/flowering and high temperature during GMP (Paper VI). In addition, a decrease in plant maturation time resulted in extensive degradation of proteins during the malting process (Paper VI). The results presented in this thesis indicate a higher breakdown rate of proteins at higher levels of TOTE and lower %UPP (Papers II and VI). However, this indication needs to be confirmed by additional investigations. It is probable that the negative influence of high GPC in malting barley on beer quality can be attributed to the higher breakdown rate of proteins at higher protein levels. A higher breakdown rate of proteins might result in higher amounts of free amino acids and peptides within the malted barley. These smaller protein-related compounds can easily be transferred into the finished beer and thus influence the beer quality.

4.6 Importance of various factors in governing grain protein concentration and protein composition

In a number of previous studies, the individual importance of G and E factors has been evaluated to determine GPC and protein composition in wheat and barley grains (Andersson & Holm, 2011; Wieser & Seilmeier, 1998; Payne & Lawrence, 1983). However, only a few studies have evaluated the importance of various factors and their relations/interactions on GPC (Johansson et al., 2003, 2001). Even fewer have evaluated the importance of various G and E factors and their relations/interactions for grain protein factors (Johansson *et al*., 2005, 2008). Of those studies evaluating more than one individual factor, most have examined the importance of genotype and environment and their interactions (Johansson *et al*., 2001, 2005, 2008). This thesis represents a first attempt to evaluate the importance and interactions of a number of various G- and E-related factors on GPC and protein composition (Papers IV and VII). By using barley, it was possible to conclude that pre-anthesis temperature is the main determinant of TOTE, while cultivar and post-anthesis temperature are of higher importance for %UPP. The growing medium used, generally, played a low role in governing the protein parameters (Paper IV). In wheat, the temperature during cultivation highly influenced the importance of various additional G and E factors on protein parameters (Papers V and VII). At high temperature during GMP, the combination of CDMT and N application timings explained 59% of the variation in TOTE, while at low temperature during

GMP only 22% of the variation in TOTE was explained by the same factors (Paper VII). The relative influence of CDMT and N application timings on %UPP was also found to be temperature-dependent. For %UPP, 50% of the variation was caused by CDMT combined with N application timings at low temperature, and only 36% of the variation was explained by the same factors at high temperature (Paper VII). Further, at high temperature, CDMT was the factor of highest relevance for determining TOTE and %UPP while at low temperature; late N application was of higher relevance.

4.7 Options for governing protein concentration and composition

As wheat and barley crops are important for growers, it would be beneficial if the GPC and grain protein composition of the grain produced could be governed using selections of G and E in order to obtain beneficial quality. Increased knowledge as to how various G and E factors can be used to govern GPC and protein composition is therefore of relevance. This thesis shows that any G and E factor that influences the plant maturation time until anthesis also influences TOTE (Papers V, VI and VII). A decrease in the length of plant maturation time until anthesis increases the amount of TOTE (Papers V, VI and VII). It seems that the various G and E factors that affect plant maturation time until anthesis, and thereby TOTE, interact with each other. Furthermore, the greater the number of factors influencing the plant maturation time in the same direction, the higher the effect obtained, although the effect was not totally additive in the present thesis work (Papers V, VII and VII). For a farmer or grower, there are thus several options available for influencing plants towards a shorter plant maturation time until anthesis if an increase in GPC (TOTE) is desired. These options include:

1) Selecting a genotype with short time to anthesis

2) Selecting a soil with not too much N availability early in the season

3) Selecting a nitrogen application regime with a relatively low dose of N applied earlier in the season.

However, a negative relationship also exists between GPC and GY and this has to be borne in mind when manipulating plant maturation time in order to increase GPC. A reduction in plant maturation time might also decrease GY.

This thesis shows that it is slightly more complicated to govern grain protein composition than GPC (Papers II-VII). The %UPP was found to be highly influenced by genotype and temperature after anthesis (Papers IV, V and VII). The results on the influence of genotype and temperature during GMP on %UPP are mainly confirmation of previous findings (Johansson *et al*., 2005). However, this thesis also shows the variation in the effects of various G

and E factors for the determination of %UPP at various temperatures (Papers IV and VII) and the dependency of %UPP on TOTE (Papers II, IV, V, VI and VII). Therefore, to govern protein composition and thereby quality, choice of cultivar has to be the first consideration for the grower, while decreased TOTE should be governed by E factors as described above to obtain increased %UPP (*e.g*. for gluten strength; Johansson, 2002; Marchylo *et al*., 1989). Furthermore, the prevailing temperature for the area of cultivation should be recorded and depending on this temperature, various E factors can be chosen. Thus, %UPP is increased if there is an increase in temperature of 7°C, *i.e*. from 17/14 °C to 24/21 °C from spike formation and in the whole GMP (Paper VII). To increase %UPP at low temperatures (*i.e*. 17/14 °C), a cultivar with long maturation time could be chosen and combined with N application at spike formation (Paper VII).

4.8 Modelling grain protein concentration and grain composition

In agricultural and plant science, crop simulation models are being used increasingly as they provide the best-known approach for integrating the understanding of complex plant processes as influenced by E factors (Sinclair & Muchow, 2001). Several investigations have been carried out on creation of simulation quality models for modelling GPC and protein composition in wheat (Martre et al., 2003, 2006; Jamieson and Semenov, 2000). Simulation models have been considered a powerful tool in investigating the individual and interactive influence of E factors on GPC and protein composition; moreover, these models also give the most powerful insights if their descriptions are mechanistic. SiriusQuality1 model (derived from Sirius model) has been used successfully to simulate GPC and protein accumulation and composition in wheat by using different E factors e.g. pre- and post-anthesis N application rate and timings, post-anthesis temperature etc. (Martre et al., 2003, 2006). However, most of the work done previously to create quality models, for modelling GPC and protein composition in wheat, involves the E factors. Not much work is done in order to simulate quality models by using combination of G and E factors in relation to GPC and protein composition. During this thesis work, some attempts have been carried out in order to model GPC and protein composition in wheat. One desire was to create a data modeling system in which the farmer should be able to submit information about the situation on the farm, about type of soil, mean temperature and precipitation for various periods of time etc. and thereafter the computer based system should suggest which cultivar and cultivation parameters to use. Although such a system would be feasible, it was not fully reached during the present thesis work. The results from this thesis can partly be used to model GPC and protein composition in wheat and barley by generating a simulation based quality model. However, additional experiments will also be needed in

order to collect enough numbers and variation in parameters to catch the pattern of the variation.

4.9 Relevance of the results for field cultivations of wheat and barley

The results from this thesis showed the importance of the G and E factors in governing GPC and protein composition in wheat and barley. However, all experiments have been carried out in controlled conditions, in green-houses and climate chambers of various types. Most likely the overall amount of results are applicable also during field conditions. However, there is a need to evaluate the main conclusions from this thesis work in field as well, to establish relationships of relevance even during such conditions. Therefore, field trials should be conducted in various climatic conditions, with varying N application and availability, using various genotypes and taking notes on plant maturation times and measurements of TOTE and %UPP.

5 Conclusions

- CDMT was found to influence TOTE and %UPP. Early maturation time of a cultivar was found related with high TOTE while late maturation time correlated with an increased %UPP. The maturation time explained variation in %UPP to a higher extent than specific protein composition did.

- Temperature before anthesis was found to influence the GPC substantially, while temperature after anthesis was found to influence %UPP. A high temperature before anthesis resulted in high GPC or TOTE while a high temperature after anthesis resulted in high %UPP.

- Soil originating from different locations played an important role in influencing the early growth stages, GY, GPC, protein composition and breakdown of proteins during malting of spring malting barley.

- Genotypes were found having a relatively high GY, combined with either low or high GPC

- From 12 DAA the variation in the speed of the build-up of TOTE and %UPP started and continued throughout the GMP. For the combinations of G and E factors resulting in high TOTE and high %UPP, at maturity, the increase was steady from 12 DAA. For the combinations of G and E factors resulting in low %UPP at maturity, no increase in %UPP could be seen during the whole GMP

- A combination of G and E factors such as CDMT, variation in temperature before and after anthesis, N amount and timing, soil and starter fertilizer showed a larger impact on GPC and protein

composition than individual influence of each of the factors. However the contribution of the different G and E factors was not straightly additive. All factors resulting in a reduced plant maturation time before anthesis contributed to a higher TOTE while the influence on %UPP was more complex.

- G and E factors influencing the plant maturation time also affected the breakdown rates of various types of proteins at malting. A higher breakdown rate of the proteins at higher levels of TOTE and lower %UPP was indicated.

- The temperature during the cultivations was largely influencing the importance of various G and E factors on the protein parameters. At high temperature CDMT was of higher relevance for determining TOTE and %UPP than at low temperature, as then late N application was of higher relevance.

- Maturation time can be manipulated and governed by the combination of agronomic and E factors and thereby the GPC and protein composition in wheat and barley.

6 Future prospects

- To investigate the combined influence of G and E factors on GY, GPC, protein build-up, composition and accumulation in field conditions for wheat and barley.

- To better understand the additive/non-additive effects of various parameters on the quality in wheat and barley.

- To evaluate importance of various soil parameters on GPC and protein composition as well as on quality.

- To investigate the combined influence of G and E factors in field conditions on protein composition and bread-making quality parameters of wheat and protein composition and malting quality parameters of malting barley.

- Based on field and controlled environment studies, a future ambition is to develop a mathematical simulation system or model for wheat and barley that can predict the GPC and protein composition by selecting the most suitable cultivar (in terms of maturation times), cultivation practices (*i.e.* sowing dates) and environmental conditions (*i.e.* N fertiliser and temperature).

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