Nitrogen Use Efficiency and Related Functional Traits in Wheat

Influence of Genotype and Environment

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Cover: *Triticum aestivum* ssp. *aestivum*, bread wheat
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Abstract

Efficient use of nitrogen (N) is essential to decrease negative impacts of agriculture on the environment. This thesis evaluated the nitrogen use efficiency (NUE) of various wheat varieties, identified traits associated with high NUE and investigated the gene *NAM-B1* in relation to some of these traits. To achieve this, a NUE concept was first developed.

Six varieties each of winter and spring wheat (*Triticum aestivum* ssp. *aestivum*) at two levels of N fertilisation were studied in a one-year field experiment. A greenhouse experiment was performed with the same six spring wheat varieties at two levels of N fertilisation and three levels of drought. Forty-two varieties of spring wheat were grown in a climate chamber experiment. Furthermore, 527 wheat accessions were genotyped for their allele type of *NAM-B1*, a gene known to affect grain N concentration and senescence of wheat.

The NUE concept developed here proved relevant in different experimental set-ups, as shown by the varieties ranking similarly in overall NUE and uptake efficiency in the greenhouse and the field. Associations between functional traits and NUE were more evident at low than at high N, suggesting that functional traits might be particularly useful for identifying efficient varieties in limiting N conditions. Grain N content was associated with chlorophyll in the top leaf around anthesis (measured by a SPAD chlorophyll meter) both in the climate chamber and in winter wheat in the field. This suggests that a high SPAD value in a variety can be used as an indicator of high N yield.

Genotyping revealed that the functional allele of *NAM-B1* has been preserved in many varieties from Denmark, Finland, Norway and Sweden, but not in varieties from other parts of the world. Swedish varieties with this functional allele did not have higher grain N concentrations, but had accelerated senescence and more ears. Effects of the allele in different environments, *e.g.* varying temperature and fertilisation, should be further investigated to determine when this allele has positive effects on grain N concentration and yield.

Keywords: Triticum aestivum L., NUE, nutrients, drought, *NAM-B1*, *GPC-B1*, senescence, spring wheat, winter wheat, early vigour.

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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 Weih, M., Asplund, L. & Bergkvist, G. (2011). Assessment of nutrient use in annual and perennial crops: A functional concept for analyzing nitrogen use efficiency. *Plant and Soil* 339(1-2), 513-520.

II Asplund, L., Bergkvist, G. & Weih, M. (2014). Proof of concept: nitrogen use efficiency of contrasting spring wheat varieties grown in greenhouse and field. *Plant and Soil* 374(1-2), 829-842.

III Asplund, L. Bergkvist, G. & Weih, M. Functional traits associated with nitrogen use efficiency in wheat (manuscript).

IV Hagenblad, J., Asplund, L., Balfourier, F., Ravel, C. & Leino, M.W. (2012). Strong presence of the high grain protein content allele of *NAM-B1* in Fennoscandian wheat. *Theoretical and Applied Genetics* 125(8), 1677-1686.

V Asplund, L., Bergkvist, G., Leino, M.W., Westerbergh, A. & Weih, M. (2013). Swedish spring wheat varieties with the rare high grain protein allele of *NAM-B1* differ in leaf senescence and grain mineral content. *PLoS ONE* 8(3), e59704.

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The contribution of Linnéa Asplund to the papers included in this thesis was as follows:

I Was involved in developing the conceptual rationale, wrote sections of the paper and commented on the text.

II Participated in designing the experiments, carried out the experiments and sampling with the guidance of the co-authors, analysed the data and wrote a large part of the paper.

III Participated in designing the experiments, carried out the experiments and sampling with the guidance of the co-authors, analysed the data and wrote the majority of the paper.

IV Carried out laboratory work on Swedish varieties, performed and wrote about the statistical analysis and commented on the text.

V Designed and carried out the experiment with the guidance of the co-authors, analysed the data and wrote the majority of the paper.

Abbreviations

N	Nitrogen
NUE	Nitrogen use efficiency
U_N	Mean N uptake efficiency
$E_{N,g}$	Grain-specific N efficiency
$C_{N,g}$	Grain N concentration at final harvest
N_s	N content of seed (sown) grain
N_g	N content of produced grain at final harvest
N'	Mean plant N content during the major growth period
B_g	Biomass of produced grain at final harvest
B	Plant biomass at final harvest
NAM-B1	*No Apical Meristem B1*
GPC	Grain protein concentration
SRL	Specific Root Length

1 Introduction

Wheat is one of the most widely grown crops in the world, together with maize and rice. For example, the total worldwide production of wheat was around 671 million tonnes on an area of 215 million ha in 2012, compared with *e.g.* production of 242 million tonnes of soybean on 105 million ha (*FAOSTAT 2014*). Wheat is therefore an important source of both carbohydrates and protein in human and livestock nutrition (Shewry, 2009). It is estimated to contribute as much protein as the total annual soybean crop, or 60 million tonnes of protein per year (Shewry, 2009). Increased nitrogen (N) fertilisation in combination with *e.g.* shorter varieties was an important factor in the increases in wheat yield achieved during the 20th century (Khush, 1999). However, there are problems associated with N fertiliser use, because N can leach and cause eutrophication of water (Vitousek *et al.*, 1997), and N fertilisation increases emissions of the greenhouse gas nitrous oxide (N_2O) from agricultural soils (Bouwman *et al.*, 2002). Volatile ammonia emissions from fertiliser contribute to deposition of N in unmanaged ecosystems (Vitousek *et al.*, 1997). Estimates on a global scale suggest that only about 50% of the N reaching agricultural fields is recovered in crops and their residues (Smil, 1999). In the UK, about 65% of available N is recovered in wheat crops and their residues (Sylvester-Bradley & Kindred, 2009) and similar recovery levels have been recorded in Australia (Fischer *et al.*, 1993). Improving N use efficiency (NUE) could therefore contribute to more sustainable agriculture.

2 Aims and hypotheses

The main aims of this thesis were to evaluate the NUE of various wheat varieties, identify traits associated with high NUE and investigate the gene *NAM-B1* in relation to some of these traits. To achieve this, a NUE concept was first developed (Paper I), with NUE components that reflect important aspects of crop growth. The hypotheses tested were as follows:

- There are differences in functional traits and in NUE components between varieties, *i.e.* there is variation associated with genotype which allows for breeding advances. Some target functional traits are leaf chlorophyll content, specific root length and leaf senescence parameters (Papers II, III, V).
- NUE is determined by a suite of functional traits (Paper III). Some specific hypotheses were:
    - High specific root length is positively associated with N uptake efficiency.
    - High leaf chlorophyll is positively associated with high N uptake efficiency.
    - Delayed leaf senescence is associated with greater grain-specific N efficiency and lower grain N concentration.
- The functional wildtype (wt) allele of the gene *NAM-B1,* which is believed to confer increased N retranslocation and accelerated senescence, has been preserved in spring wheat varieties grown in Sweden and other countries at high latitude (Paper IV).
- Varieties with the functional wt allele of the gene *NAM-B1* have faster senescence and higher concentrations of N, iron (Fe), zinc (Zn), and manganese (Mn) in the grain than varieties with a non-functional allele (Paper V).

3 Background

3.1 Nitrogen use efficiency (NUE)

Nitrogen is the mineral nutrient required in the highest concentration by plants (Taiz & Zeiger, 2006), while the amount of N available to the plant generally is limited (Elser *et al.*, 2007). Nitrogen is therefore central for plant distribution (*e.g.* Suding *et al.*, 2005), physiology (*e.g.* Evans, 1989), growth (*e.g.* Ågren, 1985) and reproduction (*e.g.* Sinclair & Jamieson, 2006) and for that reason it is the main component of agricultural fertiliser. The efficiency with which the plant utilises N, the NUE, has gained much attention. Depending on the interest of the user, this efficiency has been calculated in different ways. It should also be noted that the 'N' in the abbreviation 'NUE' sometimes also represents 'nutrient' but in this thesis NUE only refers to nitrogen use efficiency.

3.1.1 NUE in agronomy

According to Fageria *et al.* (2008), a nutrient-efficient plant is defined as one "that produces higher economic yield with a determined quantity of applied or absorbed nutrient compared to other or a standard plant under similar growing conditions". This definition highlights human use of the plant, the yield and the economic perspective, which are central in the field of agronomy. In terms of calculating NUE, one common definition used in agronomy is that by Moll *et al.* (1982), according to which NUE is grain weight (G*w*) divided by the N supply (N*s*). If total N in the plant at maturity (N*t*) is included, NUE can be further divided into at least two different components, uptake efficiency (N*t*/N*s*) and utilisation efficiency (G*w*/N*t*). Multiplying these two components yields the overall NUE (G*w*/N*s*).

Somewhat bridging agronomy and ecology aspects, Siddiqi & Glass (1981) suggest calculating the "efficiency of utilisation" as the inverse of the internal nutrient concentration times the biomass (or yield). They also suggest a ratio of utilization efficiencies to be used as a "utilization index" for comparisons of different plants (*e.g.* varieties). Similar approaches for comparing varieties have been used by others.

3.1.2 NUE in ecology

In (plant) ecology nutrient use efficiency was initially defined as the plant biomass produced per gram of nutrient in the biomass, *i.e.* the inverse of plant tissue concentration (see review by Chapin, 1980, p. 246). The time aspect was introduced with nitrogen productivity (Ingestad, 1979; Ågren, 1985), as the rate of dry matter production per unit of N in the plant in a given time (dry weight g^{-1} N day^{-1}). Vitousek (1982) developed a definition for perennial systems, suggesting that nutrient use efficiency should be the “grams of organic matter lost from plants or permanently stored within plants per unit of nutrient lost or permanently stored”. Berendse & Aerts (1987) also emphasised the losses, or perhaps one should say the retention, of nutrients. Plant populations adapted to environments differing in nutrient availability often differ in this regard and it affects the competitive ability of plant species. Berendse & Aerts (1987) proposed defining NUE as the nitrogen productivity multiplied by the mean N residence time. Under steady-state, NUE would be the “dry weight which can be produced per unit of nitrogen taken up”.

3.1.3 Differences in use of the NUE concept

In the field of ecology, the focus of NUE seems to be what the plant does with the N taken up. In contrast, in the field of agronomy there is more focus on N in the environment. Furthermore, harvested yield is not included in the ecology definitions, since yield is not directly applicable for non-cultivated plants. These contrasts highlight differences between ecology and agronomy, and partly explain why NUE definitions differ between the two fields. However, a definition that could be used in both situations would be helpful for transfer of results between the two fields of research and comparisons of different crops or systems. Such a definition is presented in Paper I, and is also briefly summarised in the Methods section of this thesis.

3.2 Plant traits related to nitrogen use

While the plant root is the major organ involved in N uptake, the whole plant utilises N. In plants, N is particularly important for photosynthesis, where it is needed for both binding CO_2 (RubisCO) and harvesting the light (chlorophyll and associated proteins) (Evans, 1989). Nitrogen is later transported to the grains. From a human perspective, grain N is important for the gluten content, which is responsible for the viscoelasticity of wheat dough that allows bread to rise (Shewry, 2009). Since N is involved in many functions in the plant, a change in one level does not necessarily result in the anticipated outcome on the plant level. For example, one study estimated the theoretical outcome of a 50 % increase in RubisCO mRNA-levels in soybean (Sinclair *et al.*, 2004). If more N was available there would only be a 6 % increase in yield, otherwise there would be a 6 % decrease. Therefore studies are needed not only on specific physiological traits, but also on plant or crop stand level.

Functional traits are defined as “morpho-physio-phenological traits which impact fitness indirectly via their effects on growth, reproduction and survival” (Violle *et al.*, 2007). This definition characterizes the traits investigated in this thesis.

3.2.1 Nitrogen uptake

Plant roots take up N mainly in the form of nitrate (NO_3^-) and ammonium (NH_4^+), but also organic N in the form of amino acids (Näsholm *et al.*, 2001; Maathuis, 2009). Since the roots are responsible for N-uptake, root morphology could affect the N status of the plant.

The root system is important for access to nutrients such as N and to water (King *et al.*, 2003; Wasson *et al.*, 2012). Specific root length (SRL) is a measure of the length of root produced from one unit of biomass. Specific root length has been found to be correlated with N uptake, *i.e.* comparing nine different species of trees (Reich *et al.*, 1998).

Early vigour is a term used to describe the growth of cereal seedlings, *e.g.* wheat, that have more vigorous early growth. It has been measured in different ways in previous studies, *e.g.* as seedling biomass (Liao *et al.*, 2004; Bertholdsson & Kolodinska Brantestam, 2009), leaf area development (Rebetzke *et al.*, 2004; Maydup *et al.*, 2012), or both (Richards & Lukacs, 2002; Watt *et al.*, 2005). In general, seedling root and shoot weight are often related. In a study on twenty varieties of Argentinian wheat, seedling root weight was found to be correlated to leaf area (Maydup *et al.*, 2012). Studies in barley using hydroponics have shown that decreasing seedling shoot weight due to breeding has been accompanied by decreasing root weight (Bertholdsson & Kolodinska Brantestam, 2009). Furthermore, varieties with higher seedling root weight (in hydroponics) have higher N uptake at low levels of N fertilisation in the field (Bertholdsson & Kolodinska Brantestam, 2009). Earlier studies found that a variety of wheat with early vigorous growth also had more vigorous roots and took up more N in pot experiments than less vigorous lines (Liao *et al.*, 2004, 2006). The same line also produced greater shoot and root biomass in unploughed soil than a conventional cultivar (Watt *et* *al.*, 2005). This suggests that early vigour might be beneficial for N uptake and, ultimately, grain biomass and grain N.

Nitrogen accumulates in the wheat plant with generally higher accumulation rates during the major growth period than during grain filling (Malhi *et al.*, 2006; Baresel *et al.*, 2008). Since uptake occurs in the root, it would be interesting to study the roots in later growth stages. That is difficult for practical reasons, but there are some data available. In a study of three Swiss spring wheat varieties grown in lysimeters with rhizotrons, the varieties differed in root growth during grain filling but there was no obvious connection with differences in the aboveground N fractions (Herrera *et al.*, 2013). Plant N uptake is also interlinked with the senescence of the plant. Some evidence from field experiments suggests that varieties with high post-anthesis N uptake deviate from the commonly observed negative relationship between grain protein concentration and yield (Bogard *et al.*, 2010). In such varieties, higher protein concentration is not associated with low yield, as is often the case.

3.2.2 Biomass production and allocation

As the plant is growing, it takes up and utilises N for photosynthesis and many other processes. During the time of active growth N is needed for biomass production, and therefore the NUE equation used in this thesis (see Chapter 4) considers the mean N during the major growth period and not only the N present in the plant at harvest.

Within plant species, the amount of chlorophyll in the leaf per unit leaf area is correlated with the total leaf N per leaf area when leaf age and N fertilisation are varied (Evans, 1989). Growth rate, or biomass production, is affected by the internal N concentration (Ågren, 1985) along with environmental factors. One method of estimating plant N content is to measure the leaf chlorophyll content. The Minolta SPAD-502 chlorophyll meter (specified in Paper V) is a tool for assessing leaf chlorophyll in a fast, non-destructive manner. It measures light transmitted by the leaf at two wavelengths and calculates a ratio (according to manual instructions). The resulting SPAD value is related to the amount of chlorophyll in the leaf, but is unitless. The SPAD value of the uppermost leaf has been shown to be strongly correlated with leaf N and whole plant N in wheat in the field around stage 37-41 and stage 52-58 (Zadoks scale, Zadoks *et al.*, 1974), with r-values between 0.78 and 0.93 (Peltonen *et al.*, 1995). The SPAD value has also been found to be correlated with grain biomass (Le Bail *et al.*, 2005).

Highlighting the close relationship between N and biomass production, it has been shown that the amount of N accumulated by the crop is closely related to the number of grains and the yield (Sinclair & Jamieson, 2006). The number of grains has been found to generally be more important for wheat yield than the weight of the single grains (Peltonen-Sainio *et al.*, 2007). Many studies indicate that the yield of wheat is in fact limited by the number of grains (Fischer, 2008 and references therein). The wheat ear initiates many florets (up to ten per spikelet), but only a fraction of these eventually develops to grains. Around four grains are commonly developed per spike, the rest of the florets are aborted prior to anthesis (Kirby, 1988). Based on experiments manipulating the photoperiod it has been suggested that increasing the length of the stem elongation could increase the number of fertile florets and thereby increase yields (Slafer *et al.*, 2001). Increasing the partitioning of assimilates to the ears at anthesis by increasing the duration of stem elongation, and by other means (*e.g.* reduced partitioning to the peduncle and other structural biomass) has been suggested as a promising route for increasing wheat yields (Reynolds *et al.*, 2009).

3.2.3 Senescence

"The process of senescence can be represented as endogenously controlled deteriorative changes, which are natural causes of death in cells, tissues, organs or organisms" (Noodén, 1988, p. 3). Senescence of plant leaves begins with structural changes in the chloroplasts of mesophyll cells, accompanied by chlorophyll degradation (Lim *et al.*, 2007). This can easily be seen and monitored, since the leaves turn yellow. In wheat, whole-plant (monocarpic) senescence takes place after anthesis and N and other nutrients in the leaves are freed and transported to the developing grain (Distelfeld *et al.*, 2014), a process which can be called retranslocation. Based on analysis of free N compounds in the grain, N seems to be transported into the grain mainly through the amino acid glutamine (Howarth *et al.*, 2008). The amino acids are then used to synthesise other amino acids and proteins. In winter wheat, the proportion of the total N content in the grain originating from N stored before anthesis is estimated to be around 70%, but the actual value is affected by the genotype and the environment (Kichey *et al.*, 2007).

Senescence is considered a complex process which can be affected by both internal factors, *e.g.* reproduction, and external factors, *e.g.* nutrient limitation or drought (Lim *et al.*, 2007). Nitrogen limitation and drought have been found to accelerate senescence in wheat. Nitrogen deficient plants complete their N remobilisation 7-14 days earlier than plants with higher N status, based on N content in the leaf (Howarth *et al.*, 2008). The proportions of different metabolic compounds in the leaf during grain filling are also affected by the environment (varying the supply of N and sulphur (S)) (Howarth *et al.*, 2008).

In contrast, the proportion of different compounds in the grain seems more dependent on plant developmental stage than the environment, as N and S treatments have a smaller effect on the composition of the grain than time after anthesis (Howarth *et al.*, 2008). According to those authors, this (lack of) coordination between senescence and grain fill could potentially be modified by breeding, to ensure optimal use of available N resources.

While environmental stresses often accelerate senescence, varieties with the ability to stay green under stress have been found to give higher yields. The 'stay-green' trait has been identified in drought-tolerant varieties of sorghum (*e.g.* Borrell *et al.*, 2000), and is also associated with higher yields at low N conditions in sorghum, maize and wheat (Borrell & Hammer, 2000; Schulte auf'm Erley *et al.*, 2007; Gaju *et al.*, 2011). In addition, the stay-green trait has been discussed as a way of increasing the yield of crops in general, through maximising the investment made in each leaf (Thomas & Howarth, 2000). However, there may be a trade-off between keeping N in the leaves to produce biomass that might be translocated to the grains, and transporting N to the grain earlier and possibly more efficiently.

Senescence of wheat leaves during grain filling is a type of programmed cell death which is controlled by the plant. In the model plant *Arabidopsis thaliana* more than 800 genes have been found to have an altered expression pattern in leaves during senescence (Buchanan-Wollaston *et al.*, 2005). A specific group of genes believed to affect plant senescence is the *NAM* (*No Apical Meristem*) genes. The *NAM* genes belong to a larger family, the NAC (NAM, ATAF1,2, CUC2) transcription factors, which are known to be important for many plant developmental processes (Olsen *et al.*, 2005). An RNAi wheat line in which expression of all *NAM* genes is repressed has been produced and it has been found that 12 days after anthesis the flag leaves of this line shows a change in expression pattern for 691 genes (Cantu *et al.*, 2011). Some of these genes are down-regulated in the line with functional *NAM* alleles compared to the RNAi line, for example some genes believed to be related to the photosynthetic machinery and other functions no longer needed during senescence. Others are up-regulated, for example proteins induced by jasmonic acid and abscisic acid. Jasmonic acid is known to be involved in plant defence against herbivores and necrotrophic pathogens, while abscisic acid is involved in many stress responses (Taiz & Zeiger, 2006; Pieterse *et al.*, 2009). Uauy *et al.* (2006a) showed that this same RNAi wheat line has delayed senescence, as well as reduced concentrations of protein, Zn and Fe in the grain. This indicates a genetic link between senescence and the retranslocation of nutrients from the senescing leaves to the grain, suggesting it could be manipulated using plant breeding.

3.3 Breeding and NUE

Wheat was domesticated around 10 000 years ago in the Fertile Crescent of South West Asia, at a time when humans were transitioning from being hunter-gatherers to being farmers. *Triticum aestivum* ssp. *aestivum*, or bread wheat, is a hexaploid species which is a hybrid with genomes from three ancestral species. It was formed when tetraploid domesticated *T. turgidum* wheat hybridised with the diploid *Aegilops tauschii* (Zohary *et al.*, 2012). The cultivation of bread wheat has been very successful and it is grown in a wide range of environments worldwide. Part of this ability to adapt to different environments may be due to the large genetic diversity contained in the three genomes, and a buffering effect of being hexaploid leading to subtle, rather than detrimental, effects of mutations (Dubcovsky & Dvorak, 2007). It has been suggested that functional traits of seed and seedlings contributed to the domestication of wheat, in preference to other wild grass species (Cunniff *et al.*, 2014). For example, grasses which were domesticated (*e.g. T. turgidum* ssp. *dicoccoides,* wild emmer) have higher seed and seedling biomass than other non-domesticated grasses also collected by early humans. These traits would give the species a competitive advantage both in the disturbed, fertile habitats in the vicinity of early settlements and in cultivated fields (Cunniff, *et al.*, 2014).

Improved yield has been an important plant breeding goal for the last 150 years or so (Åkerman, 1951; Slafer & Andrade, 1991; Sinclair, 1998; Peltonen-Sainio *et al.*, 2009). Before that, straw production for manure collection was also important and was associated with lower yield (Sinclair, 1998). However, yields of today's standard may have been reached in some societies thousands of years ago, *e.g.* in Babylon and in ancient Israel, based on information in ancient literature (Sinclair, 1998). Since yield and N are closely connected (*e.g.* Sinclair & Jamieson, 2006), it seems likely that breeding for yield has affected NUE.

Harvest index is the ratio of grain biomass to total aboveground biomass. It is a measure of the allocation of biomass between grain and straw. Early in the 20th century, the harvest index was about 0.3 (*i.e.* producing a lot of straw), whereas more recent varieties have a harvest index of about 0.5, while the total biomass has been quite stable (for a given N level) (Austin *et al.*, 1980; Sinclair, 1998). Sinclair (1998) argues that this increase in harvest index must involve increased N accumulation, since grains have a much higher N concentration than straw.

While still being much higher than straw N concentration, grain N concentration is reported to have decreased over the 20th century in both

Argentinian and Italian cultivars when varieties are grown at equal conditions (Calderini *et al.*, 1995; Guarda *et al.*, 2004).

3.3.1 NUE of wheat varieties in the Nordic countries in recent times

Nitrogen use efficiency (determined according to Moll *et al.* 1982) in Nordic spring wheat varieties has previously been shown to have increased during the 20th century (Muurinen *et al.*, 2006), mainly owing to changes in uptake efficiency. Wheat grain yields have also increased due to advances in breeding worldwide (Slafer & Andrade, 1991) and in Swedish and Finnish spring wheat varieties (Ortiz *et al.*, 1998; Peltonen-Sainio *et al.*, 2009). There is often a negative relationship between grain N concentration and yield (Kibite & Evans, 1984), but there are also exceptions. Peltonen-Sainio *et al.* (2012) studied the yield and protein concentration in Finnish cultivars and advanced breeding lines of spring cereals tested in many field trials and found a negative relationship between yield and protein concentration in spring wheat, as often observed elsewhere (*e.g.* Kibite & Evans, 1984). However, there were also lines which had both high yield and high grain protein concentration, resulting in high protein yields. In the advanced breeding lines, the protein yield was generally higher than in the official variety trials, showing that there is a real possibility to increase the protein yield.

In Sweden, breeders recognised at an early stage (in the beginning of the 20th century) that wheat landraces had some positive traits. This resulted in efforts to combine the good baking quality and early maturation of some Swedish landraces with the high yield of varieties from abroad. Early maturation was particularly important for spring wheat, which matures later than winter wheat (Åkerman, 1951).

3.3.2 Genetic background of NUE – the example of *NAM-B1*

The gene *NAM-B1* is believed to be important for wheat grain weight, grain N concentration and plant senescence. Therefore, it is related to N use and has been thought to play a role in the development of modern wheat (Dubcovsky & Dvorak, 2007). The wildtype (wt) allele of one of the *NAM* genes (see section 3.2.3), *NAM-B1*, was first identified through its positive effect on grain protein concentration (GPC) in wild emmer wheat (*T. turgidum* L. ssp. *dicoccoides*) and was initially called *GPC-B1*. Avivi (1978) observed that wild emmer had a high grain protein concentration. A locus for high GPC originating in emmer wheat was found using durum wheat (*T. turgidum* L. ssp. *durum*) - wild emmer wheat substitution lines (Cantrell & Joppa, 1991; Joppa *et al.*, 1997). The locus was later mapped with higher resolution (Distelfeld *et al.*, 2006) and the gene was sequenced (Uauy *et al.*, 2006b). The functional allele of the gene found in emmer wheat codes for a NAC-domain protein (Uauy *et al.*, 2006b). There are also two non-functional alleles, one with a one base pair insertion and one with a large deletion (Hagenblad *et al.*, 2012). The functional allele has been found to increase GPC both in tetraploid (Steiger *et al.*, 1996; Chee *et al.*, 2001; Uauy *et al.*, 2006a) and hexaploid wheat backgrounds (Mesfin *et al.*, 1999; Uauy *et al.*, 2006a; Brevis & Dubcovsky, 2010; Kumar *et al.*, 2011). Most modern wheat lines carry a non-functional allele of this gene (Uauy *et al.*, 2006b). However, the functional allele has been identified in hexaploid wheat included in a selection of wheat varieties from the International Exhibition in London in 1862 (Asplund *et al.*, 2010). This raises the possibility that the functional allele of this gene was not lost during domestication, but during modern breeding in the 20th century.

4 Methods

4.1 NUE definition

One aim of this thesis was the development of a NUE concept (Equation (1)) integrating the production aspects of agronomic NUE definitions and the plant N aspects of the ecological NUE definitions (Paper I). This requires plant sampling at several time points during plant development, which involved more work than *e.g.* the method of Moll *et al.* (1982). On the other hand, the method developed considers N during growth, which is when N is being used, rather than the situation at harvest, when N has reached its ‘final destination’ in the plant. Similarly to ecological definitions, this definition considers only internal N, *i.e.* while N in the environment affects the value, it is not included directly in the calculations. Similarly to agronomic definitions, it considers properties of the harvested plant parts. The method also allows for comparisons between *e.g.* annuals and perennials.

$$\overset{\boldsymbol{NUE}}{\frac{N_g}{N_s}} = \overset{\boldsymbol{U_N}}{\frac{N'}{N_s}} \times \overset{\boldsymbol{E_{N,g}}}{\frac{B_g}{N'}} \times \overset{\boldsymbol{C_{N,g}}}{\frac{N_g}{B_g}} \qquad (1)$$

This concept defines overall NUE as the content (*i.e.* weight) of N in the harvested grain (N_g) (sometimes called grain N yield), divided by the N content of the seed (sown) grain (N_s). In this thesis, N_s was estimated in greenhouse and field experiments by assuming that all grains that germinated had the same percentage N concentration as the N whole lot of sown grain. In the climate chamber experiment, the percentage N concentration in all sown grain was estimated by weighing sown grain and assuming a grain N concentration of 2%.

Overall NUE can be divided into three components: U_N, $E_{N,g}$ and $C_{N,g}$. U_N is the uptake efficiency, and reflects the plant's ability to use the N that was available in the seed grain to accumulate N for the plant. It is calculated by dividing N' by N_s, where N' is mean plant N content during the major growth period. In wheat the major growth period is between the start of accelerated growth in the spring and the start of grain filling (Figure 1). In practice, in this thesis these two samplings were made at a certain point in time for all varieties and treatments in each experiment, which may not always have corresponded to the actual beginning and end of the major growth period, since the varieties differ somewhat in development. It would also be possible to use a modelling approach to target the correct sampling time more accurately.

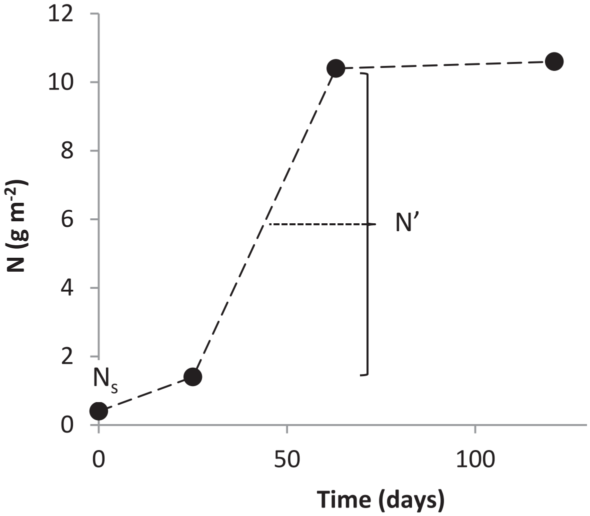

Figure 1. Example of nitrogen (N) accumulation in a spring wheat crop, indicating N amounts used in the definition of N use efficiency (NUE). N_s = N content of the seed (sown) grain, N' = mean plant N content during the major growth period.

The grain-specific N efficiency, $E_{N,g}$, is calculated by dividing grain yield (B_g) by N'. It reflects the importance of N for biomass production. The last component is $C_{N,g}$, which is the N concentration in the harvested grains. It integrates the retranslocation of N to the grain and, in the case of wheat, a quality measure.

The components can be further sub-divided for comparisons with other ecological studies, or modified for other types of crops. This is explained in Paper I, but was not utilised in any other paper in this thesis.

4.2 Summary of growth experiments

This thesis is based on data from three growth experiments (Table 1):

1. A one-year field experiment with six varieties each of winter and spring wheat and two levels of N fertilisation (Figure 2). A field experiment is the most realistic setting for a wheat crop, but it has limits because there are many factors that cannot be controlled.
2. A greenhouse experiment with the same six spring wheat varieties, two levels of fertilisation and three levels of drought (Figure 3). The experiment was performed in the greenhouse because it is easier to control irrigation under greenhouse conditions. Furthermore, using pots and perlite as a substrate allowed extreme fertilisation treatments to be included. Extreme treatments make it possible to identify effects that would be too small to measure in the field, but also limit the applicability of the results to field conditions.
3. A climate chamber experiment in which 42 varieties of spring wheat, genotyped for their allele type of *NAM-B1*, were grown without any additional treatments (Figure 4). These controlled growing conditions were used to minimize environmental variation since the effect of the allele on the phenotype was expected to be difficult to detect in varieties of different genetic backgrounds.

Table 1. *Overview of growth experiments presented in Papers II, III and V of this thesis.*

Experimental set-up	Number of varieties	Fertiliser levels	Drought levels	Number of replicates	Paper
Field	12	2	1	4	II,III
Greenhouse	6	2	3	4	II
Climate chamber	42	1	1	4	III,V

Figure 2. Field experiment with six varieties each of winter and spring wheat and two levels of N fertilisation.

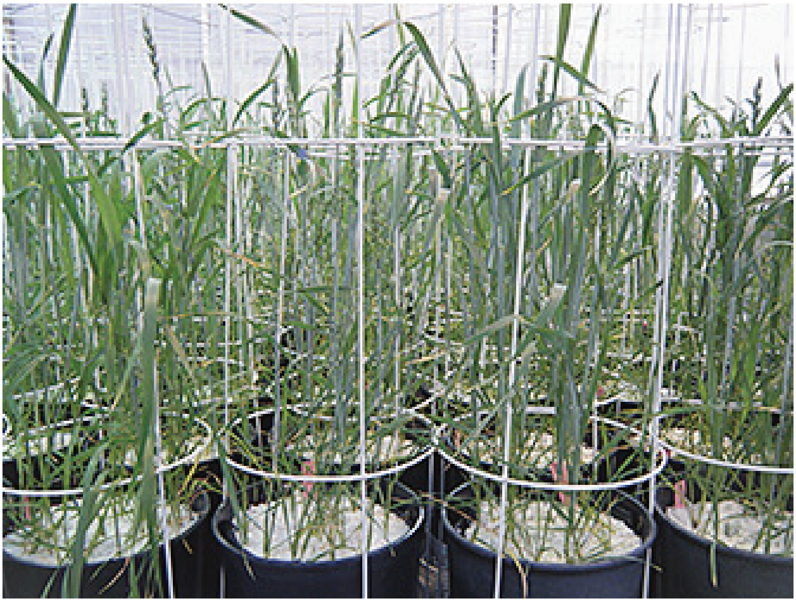

Figure 3. Greenhouse experiment with six spring wheat varieties, two levels of fertilisation and three levels of drought.

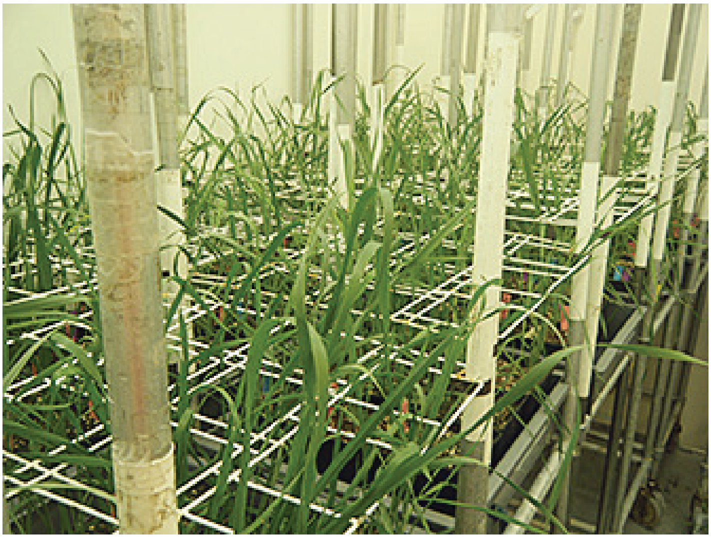

Figure 4. Climate chamber experiment with 42 varieties of spring wheat genotyped for allele type of *NAM-B1*, grown without any additional treatments.

4.3 Treatments

4.3.1 Fertilisation

Two of the experiments included N fertilisation treatments. In the field experiment, the 'high' fertilisation treatment was similar to the amount commonly used for wheat in the experimental area, which was 81 kg N ha^{-1} as ammonium nitrate, mixed with calcium carbonate and sulphur. The 'low' treatment did not receive any fertiliser and had only soil N available. In the greenhouse experiment, a complete nutrient fertiliser was used and a higher N concentration was given in the 'high' fertiliser treatment. Therefore the ratio of different elements was the same in both treatments, and plants should not have been limited by elements other than N.

4.3.2 Drought

The drought treatments in the greenhouse experiment were created by withdrawing watering until drought effects were visible and there were variations between the varieties. One treatment received full watering during the whole experiment. The second treatment had a drought that started before flowering, and therefore proceeded during flowering. The third drought treatment started when all plants had started to flower.

4.4 Plant material

For the greenhouse experiment and the field experiment, the spring wheat varieties used were ‘Diskett’, ‘Granary’, ‘Quarna’, ‘Stilett’, ‘Vinjett’, and a landrace originating from Dalecarlia (‘Dala’) that had been grown at the experimental site for around 10 generations. For the field experiment, the winter wheat varieties used were ‘Kranich’, ‘Harnesk’, ‘Kosack’, ‘Olivin’, ‘Loyal’ and a landrace originating from Germany that had also been grown at the site for around 10 generations. These varieties were chosen to span the range of variation found in varieties tested in the Swedish variety trials. The seeds were provided by SW Seed, Forsbecks AB and Lennart Karlsson, the operator of the field station where the experiment was performed.

A large set of varieties was genotyped to investigate the spatial and temporal distribution of the functional wt allele of *NAM-B1* (Paper IV). First, 367 worldwide bread wheat accessions belonging to the INRA core collection were genotyped. This collection was assembled to represent the genetic diversity present in cultivated wheat based on genetic analyses of approximately 4000 accessions (Balfourier *et al.*, 2007). Then 138 spring wheats from northerly latitudes were genotyped. These were provided by INRA Genetic Resource Center Clermont-Ferrand, the Nordic Genetic Resource Center (NordGen) and the historical seed collection of KSLA (Leino *et al.*, 2009). In addition, 22 spelt wheat accessions (*T. aestivum* spp. *spelta*) provided by INRA and NordGen were genotyped. All winter wheat varieties used in the field experiment and two of the spring wheat varieties (‘Dala’ and ‘Vinjett’) were also genotyped for presence of the functional wt allele of *NAM-B1*.

The study of grain N concentration and senescence in varieties with different alleles of *NAM-B1* included 41 varieties (Paper V), most of which were also included in Paper IV. The varieties have been grown in Sweden at some point, and/or been used as breeding material in early breeding programmes. Three of the varieties used were provided by Lennart Karlsson and 38 by NordGen.

5 Results

5.1 Effects of genotype and environment on response variables

5.1.1 Effects of genotype and environment on NUE

Overall NUE and $C_{N,g}$ differed between varieties in all three experiments. The components U_N and $E_{N,g}$ differed between varieties in the greenhouse and the field experiments, *i.e* when measured.

There were also some general differences between spring and winter wheat, based on the results from the field experiment. NUE and U_N were higher in winter wheat than in spring wheat, $C_{N,g}$ was higher in spring wheat and $E_{N,g}$ did not differ between spring and winter wheat types.

High fertilisation treatments increased NUE, U_N and $C_{N,g}$ and decreased $E_{N,g}$ in both the greenhouse and field experiments. The effect of fertilisation on NUE and components was different in different varieties in both the greenhouse and field experiments, *i.e.* there were significant genotype × environment effects. Uptake efficiency increased less in 'Granary' than in other varieties in the high N treatment in the greenhouse. The variety 'Quarna' had a smaller reduction in $E_{N,g}$ than the other varieties at high N in the greenhouse. In the field, there were mainly G × E effects in the winter wheat varieties. These effects in both NUE and the components were likely due to a high response to high N in 'Kosack', and a low response in 'Olivin'.

Drought treatments in the greenhouse decreased NUE and $E_{N,g}$, while they increased $C_{N,g}$. Drought affected the $C_{N,g}$ and NUE of different varieties in different ways in the greenhouse (*i.e*. G × E interaction).

A comparison of the NUE concept (Equation (1)) in different experiments showed that while some components differed, the varieties ranked similarly in overall NUE and U_N in the greenhouse and in the field. U_N was higher in the field and $E_{N,g}$ was higher in the greenhouse. Mean NUE was around 300 $g\ g^{-1}$ in the climate chamber experiment, which was much higher than in the other two experiments where mean NUE was around 10-40 g g^{-1} (Table 2). The NUE calculated according to Moll *et al.* (1982) was similar in the field at high N and in the climate chamber (Table 2).

Table 2. *Mean values of NUE in spring wheat in the greenhouse, field and climate chamber experiments, calculated according to the method of Weih* et al. *(2011) and Moll* et al. *(1982). The values for the greenhouse include only the treatments without drought. B_g = biomass of produced grain at final harvest.*

Experiment	N treatment	NUE_{Weih} (g g^{-1})	B_g	NUE_{Moll} (g g^{-1})
Greenhouse	low N	16	0.73 g $plant^{-1}$	38.4
	high N	44	0.86 g $plant^{-1}$	5.7
Field	low N	12	266 g m^{-2}	28.1
	high N	19	384 g m^{-2}	21.8
Climate chamber		300	22 g $plant^{-1}$	18.6

5.1.2 Effects of genotype and environment on functional traits

The varieties differed in many of the functional traits measured. Winter wheat seedlings differed in aboveground biomass and specific root length in the field experiment, but we did not find differences in spring wheat in the field. In the greenhouse on the other hand, the same spring wheat varieties' seedlings differed in SRL ($p=0.0003$) and biomass ($p<0.0001$) at the harvest in the beginning of the major growth period (GS 13, Tottman, 1987). The development around flowering differed between varieties in all three experiments. The SPAD-value of the flag leaf around flowering differed between varieties in all experiments, and the spring wheat varieties ranked similar in SPAD value in the greenhouse compared to the field. Straw biomass (at final harvest) differed between varieties in the greenhouse ($p<0.0001$) and the climate chamber ($p=0.0004$) but not in the field experiment. Senescence, measured as a visual assessment of yellow leaf area in the field experiment and modelled based on SPAD readings in the climate chamber ($p<0.0001$ for start and length of senescence) also differed between varieties.

SPAD-value of the flag leaf at anthesis was higher in the winter wheat than in the spring wheat. Senescence differed between types, mainly due to a significant type × fertilization effect where winter wheat of low fertilization had senesced more at the times of assessment (spring wheat was assessed one week after winter wheat).

High fertilization increased SPAD values in the field experiment, but not in the greenhouse ($p=0.18$ for variety × fertilization). In winter wheat there was a variety × fertilization effect of SPAD around anthesis and number of harvested grain.

5.2 Associations between NUE and functional traits

5.2.1 Multivariate analysis of NUE and functional traits

We used a partial least squares analysis to determine which functional traits that were important for NUE in the climate chamber experiment. SPAD-value of the flag leaf at stem elongation, number of grains per ear, number of ears per pot, straw biomass per pot, days between emergence and anthesis, and days between emergence and completed senescence were identified as important traits. Traits that were included in the analysis but not identified as important were single grain weight, plant height, days between anthesis and start of senescence, rate of senescence, SPAD at anthesis, harvest index, days between start and end of senescence and year of release of the variety.

5.2.2 Uptake efficiency and early vigour

Uptake efficiency was not correlated with seedling biomass or SRL in winter wheat, and spring wheat varieties in the field did not differ in these traits. There were however negative correlations between NUE and seedling biomass at low N. In the greenhouse, where differences between varieties in seedling SRL and biomass were detected, we found no significant correlations with U_N either.

5.2.3 Uptake efficiency and SPAD

There was no correlation between U_N and SPAD in the field or in the greenhouse ($p=0.06$ and $p=0.09$ for LN and HN in the greenhouse respectively). Neither were there any significant correlations between B_g and SPAD within or across treatments in the field experiment. However, there was a positive correlation between B_g and SPAD in the climate chamber experiment (Pearson correlation coefficient=0.64, $p<0.0001$). In the greenhouse experiment in the no drought treatment, there was a negative correlation between B_g and SPAD value around anthesis (Pearson correlation coefficient=-0.81, $p=0.05$) at LN, but not at HN (Pearson correlation coefficient=0.70, $p=0.12$).

5.2.4 Grain N content and SPAD

NUE was positively correlated with SPAD at anthesis in winter wheat low N. As previously mentioned, the SPAD value was also identified as important for NUE in the climate chamber experiment. In the field, N_g was positively correlated with SPAD at anthesis, in both HN and LN in the winter wheat. The correlation between N_g and SPAD was also significant in the climate chamber (Figure 5). When comparing the results from the different experiments of the spring wheat varieties grown in greenhouse and field, there was a positive correlation in NUE and U_N at both fertiliser levels, in SPAD at low N, but no correlation within any N treatment in N_g (Figure 6).

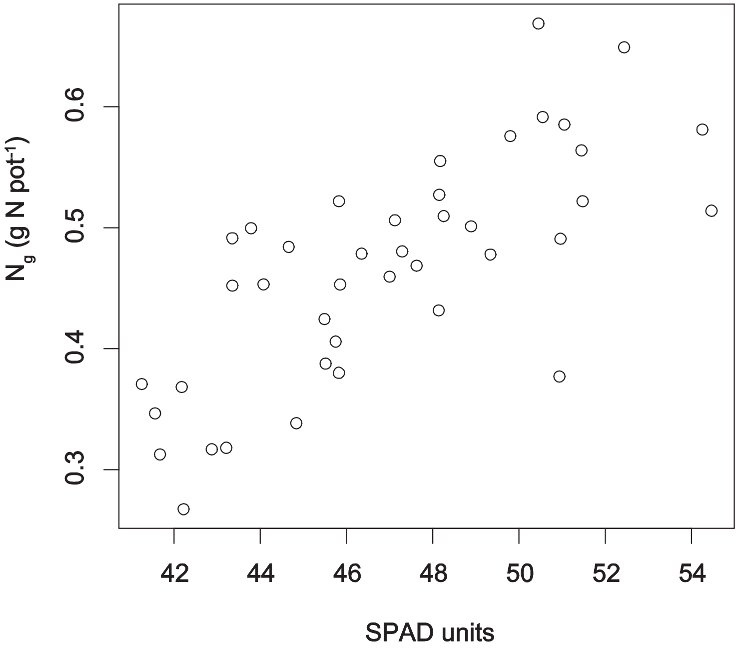

Figure 5. Plot of grain N content versus SPAD value in 41 spring wheat varieties grown in the climate chamber experiment presented in Paper III and V (Pearson correlation efficient=0.74, p<0.0001).

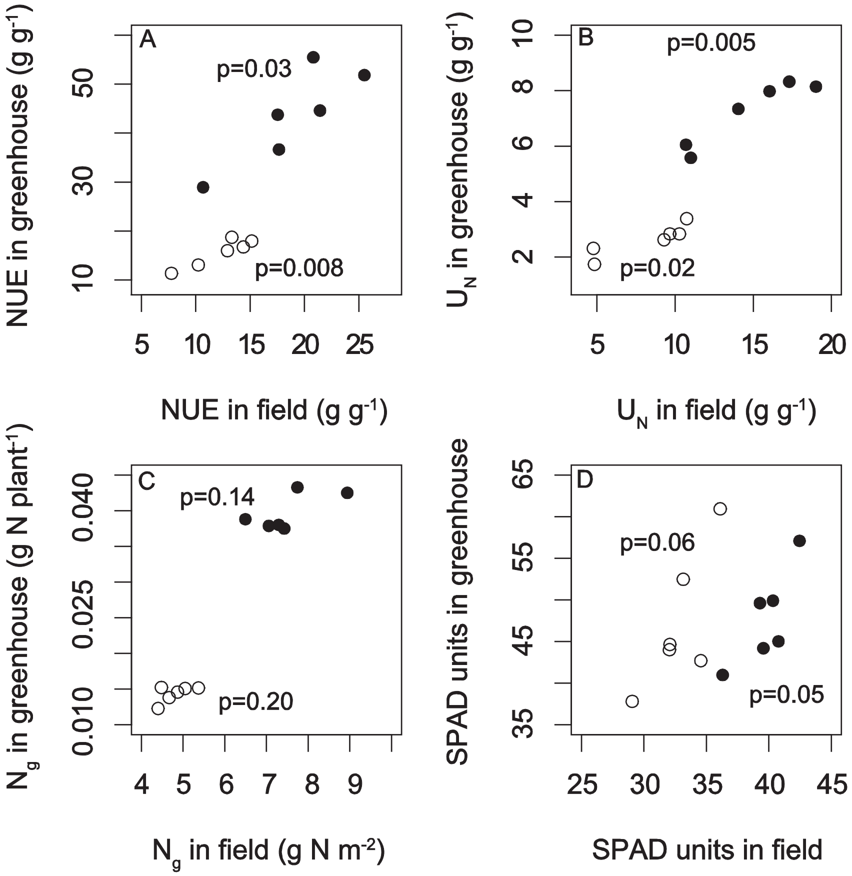

Figure 6. Scatterplots of A) NUE, B) U_N, C) N_g and D) SPAD of spring wheat varieties in the greenhouse (Paper II) and in the field experiment (Paper II, III). Symbols are mean values for each variety within the N treatments. Open circles represent the low N treatment, filled circles the high N treatment. The p-values for the correlations between values within each treatment are written in the plots.

5.2.5 Grain N concentration, grain-specific N efficiency and senescence

We found a positive correlation between yellow leaf area and $C_{N,g}$ as hypothesized, but only in winter wheat grown at LN. There was also a negative correlation between $E_{N,g}$ and yellow leaf area in the same treatment. However, NUE was not correlated with yellow leaf area in any of the treatments in the field experiment. In the climate chamber analysis the total time from emergence to complete senescence was found important for NUE but not the senescence start, rate and length. There was no significant correlation between $C_{N,g}$ and any senescence parameter, or the time from emergence to complete senescence, in the climate chamber experiment.

5.3 Distribution and possible effects of the wildtype allele of *NAM-B1*

5.3.1 Geographical distribution of the *NAM-B1* wildtype allele

The functional wt allele of *NAM-B1* was found in 5 varieties in the INRA collection of 367 worldwide wheat varieties. Out of these 5 varieties 3 spring wheat accessions could be traced to northern locations (with Polish, Finnish or Swedish spring wheats in their pedigrees). The wt allele frequency was higher in the spring wheat varieties (4/119) compared to the winter wheat varieties (1/234). Therefore, an additional 138 spring wheat varieties of northern latitude were screened for the allele of *NAM-B1*. The wt allele was found in 46 of the varieties with a northern origin (Table 2), having a frequency of 33.3 % in the northern accession compared to 1.4 % in the INRA world core collection. In this northern set of varieties, all accessions carrying the wt allele were from Denmark, Finland, Norway and Sweden. Out of the 49 Swedish accessions, 14 had the wt allele. The highest proportion of the wt allele was found in Norway. Out of 20 genotyped Norwegian varieties, 16 had the wt allele (Table 3). None of the genotyped varieties from the field experiment carried the functional allele.

Table 3. *Distribution of NAM-B1 allele type in spring wheat accessions with a northern origin. Published in Paper IV (Hagenblad* et al.*, 2012).*

	+ 1 bp	Deletion	Wildtype	Total
Total	23	69	46 (33.3 %)	138
Country				
Canada	1	8	0	9
Denmark	2	2	2 (33.3 %)	6
Finland	6	9	14 (48.3 %)	29
Germany	0	4	0	4
Japan	2	6	0	8
Republic of Korea	1	0	0	1
Mongolia	1	1	0	2
Norway	1	3	16 (80 %)	20
Poland	3	0	0	3
Russia	3	4	0	7
Sweden	3	32	14 (28.6 %)	49

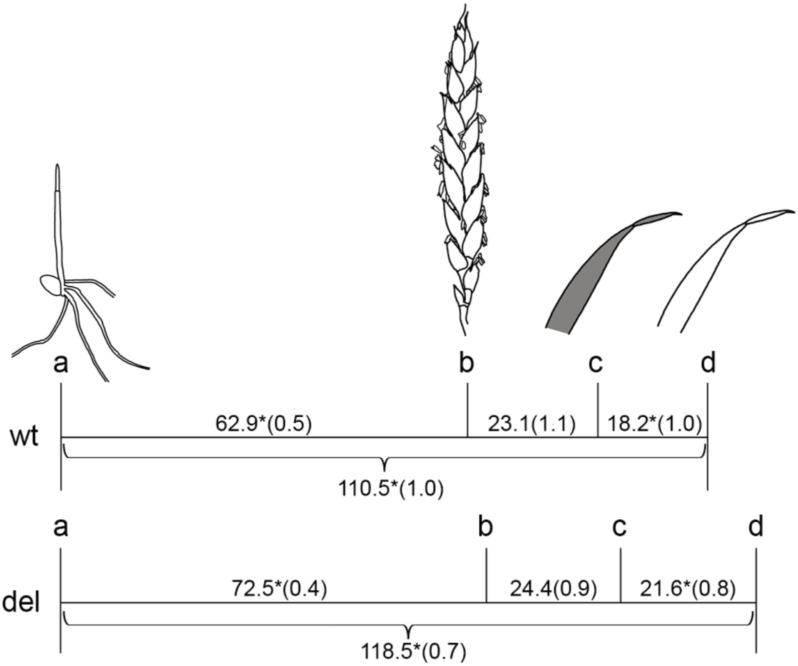

Figure 7. Timeline of development of varieties with the functional wildtype (wt) allele and varieties with the non-functional deletion (del) allele. The scale is mean number of days (SE in brackets). Time spans that are significantly different between the allele types are indicated with * on both groups of varieties ($p<0.001$). a=emergence, b=anthesis, c=modeled start of senescence of flag leaf (t), d=complete senescence of flag leaf. Published in paper V (Asplund *et al.*, 2013, doi:10.1371/journal.pone.0059704.g002).

5.3.2 Phenotypic differences between varieties carrying different alleles of *NAM-B1*

Varieties carrying the functional wt allele of *NAM-B1* had a shorter period of senescence than varieties with the non-functional deletion (del) allele (Figure 7). The wt varieties also reached anthesis earlier. However, the duration in time between anthesis and start of senescence did not differ between varieties with the wt allele compared to varieties with the del allele, resulting in a shorter total time from emergence to complete senescence in varieties with the wt allele.

The mean grain weight was lower in varieties with the wt allele compared to varieties with the del allele, but the wt varieties yielded more in total. The wt varieties had more ears, but the number of grains per ear did not differ between varieties with the wt allele compared to varieties with the del allele.

The yields (total weight per pot) of macronutrients Mg and P in the grain were higher in the group of varieties with the wt allele. The yields of macronutrients N, K, Ca and S in the grain did not differ between varieties of the different alleles. In the straw the yields of Ca and S were lower in the wt varieties.

We also studied the micronutrients (Fe, Mn, Zn, Cu, and Na). The yields of the micronutrients in the grain did not differ between the groups, but the yield of Fe in the straw was higher in the group of varieties with the wt allele.

6 Discussion

6.1 Genetic and environmental influence on NUE and functional traits

6.1.1 Differences between varieties (Papers II and III)

Overall NUE and measured NUE components differed between varieties in the greenhouse, field and climate chamber experiments (Papers II, III), which confirms that there is a genetic component of NUE which allows for breeding.

Functional traits also differed between varieties. Winter wheat seedlings differed in early biomass and SRL in the field experiment (Paper III), while spring wheat seedlings showed little genetic variation and only differed significantly in the greenhouse (Paper II). This suggests that differences between the spring wheat varieties are so small that they are difficult to detect in the field due to high environmental variation. It is also possible that the field environment influenced growth in such a way that differences between the seedling characteristics of varieties were minimised.

The developmental rate differed between the varieties studied. Therefore, since all varieties were sown on the same date, anthesis took place at different dates. It is known that the effects of environmental conditions during a specific time period on *e.g.* yield and yield components depend on the developmental stage of the crop that period. For example, high temperatures during early ear development may reduce the number of spikelets per ear and warm temperatures around anthesis may reduce the number of grains through induction of pollen sterility and infertile florets (Porter & Gawith, 1999). Since the development differed between varieties, the specific environment at the same growth stage was not exactly the same for all varieties in the field and greenhouse experiments, where conditions could not be completely regulated (Papers II and III). The specific effects of developmental rate cannot be distinguished in these experiments.

The fact that the varieties ranked similarly in SPAD values between the greenhouse and the field (Papers II and III), despite quite different growing conditions, highlights the effect of the genetic component of this plant characteristic. Senescence differed at low N in the field in winter wheat (Paper III). This could indicate that N was limiting to different extents in these varieties and/or that there were genetic differences in senescence. The functional allele of *NAM-B1* was not present in any of the winter wheat varieties or in any of the genotyped spring wheat varieties in the field. Therefore, this particular gene was not responsible for the differences between the senescence of winter wheat varieties at low N.

6.1.2 Differences between spring and winter wheat (Paper III)

The higher NUE in winter wheat than in spring wheat in the field experiment (Paper III) can partly be explained by the different seed rates. The N content of seed grain (N_s) was higher in spring wheat, while the N content of the harvested grain did not differ between spring and winter wheat. Winter wheat thus achieved the same N_g with lower input of N in the sown grain. The higher U_N in winter wheat could also be explained by larger root systems, which contribute to obtaining the N. Winter wheat roots have been observed to reach depths twice those of spring wheat (Thorup-Kristensen *et al.*, 2009). Furthermore, the SRL of winter wheat in the spring was higher than in spring wheat.

Winter and spring wheat did not differ in $E_{N,g}$ (Paper III). The $E_{N,g}$ can be viewed as a product of allocation of biomass to the grain and the total biomass produced from the N', both of which only differed between varieties and not between types (unpublished data). This indicates an absence of general differences in photosynthesis per unit of N between them. The $C_{N,g}$ was higher in spring wheat than in winter wheat (Paper III), as has been observed previously (*e.g.* in the Swedish wheat variety trials; Larsson *et al.*, 2012). This could be the result of environmental, rather than genetic, differences between the types, since winter wheat is sown several months before spring wheat and their developmental stages do not coincide. The genetic difference between spring and winter wheat was eliminated in a study investigating the same facultative wheat sown in autumn and in spring in Turkey (Ozturk *et al.*, 2006). They found that this variety had higher grain yield if sown in autumn and higher GPC if sown in spring. However, studies comparing spring and winter wheat $C_{N,g}$ for wheat grown in the same experimental design and at the same N fertiliser rate, as was done in this thesis, are rare. Since N_g did not differ between the types, while B_g was higher in winter wheat, the results are consistent with the commonly observed negative relationship between B_g and $C_{N,g}$ (Kibite & Evans, 1984; Peltonen-Sainio *et al.*, 2012).

6.1.3 Influence of N and drought treatments (Papers II and III)

High N fertilisation increased NUE in both the greenhouse (Paper II) and the field (Papers II and III). This was expected, because with higher N supply more N can be taken up and allocated to the grains. However, when NUE is calculated according to Moll *et al.* (1982) (*i.e.* grain weight/N supply), higher N fertilisation normally results in lower NUE (*e.g.* Moll *et al.*, 1982). That is because the yield generally does not increase to the same extent as the N supply (*e.g.* Holford *et al.*, 1992). A difference between varieties in NUE according to Moll *et al.*(1982) at a certain level of N fertilisation is basically a difference in grain yield, and not in N in both sown and harvested grains as in the NUE presented in Paper I. The U_N also increased at high N and this was expected, since high N availability should increase the mean N of the plant. It is well known that $C_{N,g}$ increases at high N and N fertiliser application is used to increase grain protein concentration (Hay & Porter, 2006, p. 206). The only NUE component that decreased at high N, in both the greenhouse and field experiments, was $E_{N,g}$. This was related to a decrease in total biomass produced per mean N during the major growth period. The proportion of biomass allocated to the grain was unaffected by N fertilisation in the field. The component $E_{N,g,}$ is similar to the utilisation efficiency of Moll *et al.* (1982), *i.e.* grain weight/total N at maturity. The difference is that the utilisation efficiency is based on the total plant N at crop harvest and the grain-specific N-efficiency is based on the mean N during the major growth period.

Wheat production is limited by water in many parts of the world (Araus *et al.*, 2002), and water can also be limiting at high latitudes such as Sweden and Finland (Flink *et al.*, 1995; Peltonen-Sainio *et al.*, 2011). More severe summer droughts can be expected in large parts of Sweden in the future, because of climate change (Swedish Commission on Climate and Vulnerability, 2007). The drought treatment in the greenhouse experiment (Paper II) resulted in increased grain N concentrations. Similarly, Ozturk and Aydin (2004) found that early, late or continuous drought increased GPC and decreased yields in one wheat cultivar grown in a field experiment in Turkey. The decrease in yield is mainly due to a reduction in fertile florets and grain numbers at early (pre-anthesis) drought, and a reduction of single grain weight at late (post-anthesis) drought (Rajala *et al.*, 2009). High N fertilisation actually reduced yield compared with low fertilisation in the greenhouse experiment, and NUE did not increase much at high N fertilisation in the drought treatments. Similarly, reduced grain yields at high fertilisation have been observed previously in terminal drought conditions in the field (van Herwaarden *et al.*, 1998). In that case, high early N-availability promoted vegetative growth, which resulted in a water deficit and reduced assimilation after anthesis (van Herwaarden *et al.*, 1998).

6.1.4 Influence of experimental set-up (Papers II, III and V)

In the climate chamber experiment (Papers III and V), NUE was much higher than in the field (Papers II and III) or the greenhouse (Paper II), which could be explained by the plant density in the climate chamber being much lower (2 plants pot^{-1}=151 plants m^{-2} in the climate chamber compared with 550 plants m^{-2} in the greenhouse experiment and field).

The $E_{N,g}$ was higher in the greenhouse than in the field. This was related to both a higher proportion of biomass being allocated to grain in the greenhouse (maximum mean within fertiliser treatments was 0.54 in the greenhouse and 0.40 in the field), and higher biomass production per mean N in the major growth period. However, the latter could be related to earlier sampling time in the greenhouse than in the field, and therefore the value of the comparison of absolute values is limited. The harvest index, on the other hand, could be connected to the growth conditions, since wind or other uncontrolled effects in field conditions may have contributed to sturdier plants and higher allocation of biomass to the straw in the field. The U_N was higher in the field. This could also be related to the timing of the sampling, since early sampling, as in the greenhouse, should result in low U_N and high $E_{N,g}$. It could also be connected to a difference in N availability in the field compared to the greenhouse.

It should also be noted here that plant density was used as a covariate in the analyses of NUE components in Paper II, but not in Paper III. That is why the F and p values do not correspond completely. The reason why plant density was not used as a covariate in Paper III was that both winter wheat and spring wheat were analysed in the same analysis. Since the number of plants was very different between the types, it was not appropriate to use it as a covariate. However, in general this did not change the outcome of the analyses.

6.2 NUE as determined by functional traits

6.2.1 Difference in senescence at low N – functional traits in limiting conditions (Paper III)

There was a positive relationship between the senesced leaf area and $C_{N,g}$, and a negative relationship with $E_{N,g}$ as hypothesised, in winter wheat at low N (Paper III). In addition, there were significant interactions between genotype and fertilisation in many variables in the winter wheat, but we found no such interactions in the spring wheat. These results suggest that in the field experiment, N was a limiting factor in the winter wheat low N treatments. Furthermore, there was genotypic variation due to this limitation and the functional traits studied in Paper III became a factor which separated the varieties in this situation. It has been suggested that selection in the target environment in breeding programmes promotes high yields under these conditions (*e.g.* low-input barley cultivation, Ceccarelli, 1996; low N-input wheat cultivation, Brancourt-Hulmel *et al.*, 2005; organic wheat cultivation, Löschenberger *et al.*, 2008). In line with this, the results of the field study (Paper III) indicate possibilities of measuring functional traits in the breeding for more efficient varieties in N-limiting conditions.

The genotype × environment interactions which were identified in the experiments indicate that the climate chamber experiment, which was performed under rather high N fertilisation, may have yielded different results under lower fertilisation (Paper III). At lower fertilisation levels, functional traits that were more important for NUE under limiting conditions could have been identified. However, the design used in this thesis should be more optimal for identifying traits that are important for NUE under less limiting conditions, which is relevant under prevailing agricultural management.

6.2.2 NUE is positively related to number of grains (Paper III)

The PLS identified both number of ears and number of grains per ear as important for NUE (Paper III). Together these variables determine the number of grains produced. The NUE can be viewed as how many times the N in the seed grain is multiplied in the harvested grain. Furthermore, the number of grains produced and the yield can be considered a result of the N accumulation by the plant, *e.g.* the N available in the developing spike is correlated with the number of grains (Sinclair & Jamieson, 2006). Therefore, it is logical that the number of grains produced is not only important for determining the yield, but is also related to the NUE, calculated according to Paper I. The experiments in Paper III did not allow any conclusions to be drawn regarding whether many grains result in high NUE, or the other way around. However, the results imply that breeding efforts improving yield by increasing the number of grains should also result in higher NUE, calculated as in Paper I (as well as according to Moll *et al.,* 1982).

6.2.3 Specific root length and uptake efficiency (Paper III)

The absence of an association between specific root length and uptake efficiency in the field experiment (Paper III), contradicting the initial hypothesis, could be related to effects of other root traits which were not measured. It is also possible that root traits and root distribution in the soil profile in later growth stages are more important than at the seedling stage, and these were not measured. Moreover, no connection was found between early biomass and U_N winter wheat in the field experiment or in spring wheat in the greenhouse. Early vigour is believed to be beneficial for yield for different reasons, *e.g.* increased N uptake and weed competition (Richards & Lukacs, 2002; Watt *et al.*, 2005; Bertholdsson & Kolodinska Brantestam, 2009), and further experimentation is needed to determine the relationship between seedling traits and NUE.

6.2.4 Positive relationship between uptake efficiency and grain biomass (Paper III)

The uptake efficiency (U_N) is related to the mean N in the plant during the growing season, which is likely to be important for biomass production. Accordingly, we found a positive relationship between U_N and grain biomass in winter wheat at low and high N, while across treatments the relationship was close to significant (Paper III). The relationship between the specific leaf N content (here assessed by SPAD) and B_g was positive, but not significant. Semenov et al. (2007) predicted in a modelling study that a higher specific leaf N in wheat would result in lower NUE *sensu* yield per mineral N supply, rather than higher yield as in Paper III. However, the model contained assumptions which do not necessarily hold for Paper III. The SPAD values did not fully reflect U_N in Paper III, since we did not find a relationship within treatments between SPAD and U_N. This could be related to the particular increase in leaf area in response to plant N (or U_N). Furthermore, we found no significant relationship between SPAD and the yield per mean N during the major growth period ($E_{N,g}$), probably because the different varieties allocate different proportions of the total biomass to the grains.

6.2.5 Positive relationship between SPAD value and grain N content (Paper III)

A significant positive relationship was found between the SPAD values of the uppermost leaves and grain N content. This pattern was present in the dataset of 41 varieties tested in the climate chamber, in the field experiment as a general trend over all data, and within fertilisation treatments in winter wheat. While the SPAD value at stem elongation, but not at anthesis, was found to be important in the climate chamber, the SPAD value at anthesis was correlated with NUE and N_g in the field. That inconsistency could be related to extended access to N for the plants in the climate chamber experiment compared with those in the field experiment. The SPAD values have previously been found to be correlated to protein yield within varieties of winter wheat and spring barley (Le Bail *et al.*, 2005; Spaner *et al.*, 2005). In Paper III a relationship was found between SPAD value and N_g across different varieties. These results suggest that a variety with a high SPAD value is more likely to have high N_g and NUE than a variety with a lower SPAD value, information that could be used within breeding for development of more N use efficient varieties.

6.3 Geographical distribution of *NAM-B1* and plant development (Paper IV and V)

Genotyping of a large number of wheat genotypes confirmed that the functional allele of the gene *NAM-B1* has been preserved in Swedish, Danish Finnish and Norwegian varieties (Paper IV). However, this allele was not present in varieties from all northern regions investigated here. Therefore the hypothesis that the functional allele is preserved at northerly latitudes in general was not supported. However, it still seems likely that part of the reason that the functional allele is preserved in Fennoscandia is because it is associated with traits that are beneficial in a short growing season.

Varieties with the functional allele of *NAM-B1* had faster senescence than varieties with a non-functional allele, in line with our hypothesis (Paper V). Furthermore, varieties with the functional allele had a shorter time between emergence and anthesis, as well as between emergence and complete senescence. These results suggest a possible effect of *NAM-B1* on plant developmental rate. Barley carries an ortholog of *NAM-B1* with similar effects, *HvNAM-1* (Parrott *et al.*, 2012). A barley line with the high GPC allele of this gene has been found to flower 5 days earlier than a low GPC line, an effect caused by accelerated development of the inflorescence in the high GPC line (Lacerenza *et al.*, 2010). Further investigations have shown that the high GPC barley line has earlier anthesis under long and short days, but not after vernalization, while senescence is accelerated under all conditions (Parrott *et al.*, 2012). Both accelerated senescence and early anthesis should be beneficial in an environment with a short growing season. If development in general is affected by *NAM-B1*, this could partly explain why the wt allele is so much more common in the Fennoscandian wheat varieties tested in this thesis than in the world wheat core collection. However, studies on vernalization and day length in wheat are needed to clarify this point.

Some contradictory conclusions regarding *NAM-B1* and growing season have emerged from studies of the functional allele introgressed into hard red spring wheat varieties from the Pacific Northwest Region of the USA (Carter *et al.*, 2012). While those authors found effects of the allele on senescence and grain

N concentration in greenhouse studies, such effects were not seen in the field. The temperatures during the course of their experiments (the accumulation of growing day degrees) differed between the environments, *e.g.* with high temperatures in the field after anthesis. Therefore, Carter *et al.* (2012) suggest that in the field, senescence may be already initiated before the effect of the *NAM-B1* allele is manifested. Those authors also suggest that the potential for using the functional allele of *NAM-B1* may be limited in areas with a short growing season and high mid-season temperatures. The effect of temperature regime has since been studied in an experiment with an RNAi line, in which all *NAM*-homologues are non-functional, and a functional line (Guttieri *et al.*, 2013). The functional and non-functional lines differed in patterns of senescence and grain N content both in heat stress and optimal temperature regimes and genotypes responded similarly to post-anthesis drought (Guttieri *et al.*, 2013). This suggests that the effect of these *NAM*-genes is retained under drought and heat stress.

Senescence is affected by both genotype and environment, and that could explain the difference in the effect of *NAM-B1* in different environments. However, further experimentation with *e.g.* day length, temperature, fertilization and drought is required to identify the environments in which presence of the functional allele of *NAM-B1* is beneficial.

6.4 Varieties which differ in allele type of *NAM-B1* also differ in senescence and other functional traits (Paper IV and V)

The design of the experiment described in Paper V allowed conclusions to be drawn about how varieties with a functional *NAM-B1* allele differ from varieties with a non-functional allele type. However, it is not possible to attribute this information directly to effects of the wt allele since the studied varieties also differ in other genes and the expression of the *NAM-B1* gene may be modified by other interacting genes and/or linked to other traits. However, from a breeding perspective it is still interesting to evaluate the effect of the gene in different genetic backgrounds.

Varieties with the functional allele of *NAM-B1* did not differ in grain N concentration or yield, contrary to our hypothesis and previous reports on the effect of *NAM-B1*. This may be due to the gene having only a small (and undetectable in this dataset) effect on grain N. It may also be related to the specific environment in which the varieties were grown in this thesis. Both alternatives are important to investigate further and to consider for any breeding activities involving marker-assisted selection based on variation of this gene.

There were no differences between groups of varieties in terms of straw N concentration or allocation of N between grain and straw (N harvest index, NHI). However, the high GPC allele introduced into Argentinian wheat is reported to reduce the straw N concentration and increase NHI (Tabbita *et al.*, 2013). Waters *et al.* (2009) studied N allocation and found that functional *NAM* alleles seemingly increases the efflux of nutrients from the leaves and increases the allocation of nutrients to the grain. Our varieties differed in many aspects, *e.g.* harvest index, which complicates the investigations of translocation. This issue is probably best examined by more detailed studies of nutrient allocation in lines which only differ in the allele type of *NAM-B1*.

In this thesis, the wt varieties had lighter grains but a higher total grain yield than varieties with a non-functional allele. Lighter grain in wt varieties has been noted previously (*e.g.* Tabbita *et al.*, 2013). However, the effect of the functional allele on grain weight and yield has been found to vary between experiments and genetic background of the varieties (Uauy *et al.*, 2006a). For example, one study investigated a wheat line in which all functional *NAM-1* homologues were mutated to become non-functional (Avni *et al.*, 2014). It was found that although the mutated line had delayed senescence and was photosynthetically active for a longer time (*i.e.* the stay-green trait) there were no consistent effects on grain weight or yield.

In this thesis, varieties with the functional allele of *NAM-B1* also had more ears. Such an effect of the functional allele has been observed in a few previous cases. For example, some Indian lines with the functional allele produced more tillers (Kumar *et al.*, 2011) and two Argentinian wheat varieties had more ears than varieties with the non-functional allele (Tabbita *et al.*, 2013). Higher number of ears as was recorded in this thesis may be an effect of the wt allele that is only expressed in certain varieties or environments. It is also possible that the wt allele is linked to, or controlled by, other genes in the studied Swedish varieties. More ears is a way of increasing the number of grains per unit area (Peltonen-Sainio *et al.*, 2007), and therefore a higher number of ears could contribute to higher NUE.

Spelt wheat accessions were also genotyped, and the functional allele was found in five genotypes out of 22 tested (Paper IV). These were from Iran, Turkmenistan, Belgium and Switzerland, and could not be traced to a northern origin. Spelt wheats are known for having a good baking quality, being higher in GPC and lower in yield compared to bread wheat, even though there is also variation between varieties as in bread wheat (Rüegger *et al.*, 1993; Campbell, 1997). However, we did not study spelt wheat in the climate chamber, and the effects of *NAM-B1* on senescence and nutrient retranslocation of spelt wheat remain to be investigated.

7 Conclusions

Based on the experiments presented in this thesis, the following conclusions can be drawn:

- The presented NUE concept is relevant in different experimental set-ups, shown by the varieties ranking similarly in overall NUE and uptake efficiency in the greenhouse and the field experiment.
- Associations between functional traits and NUE were more evident at low N than at high N. This suggests that functional traits might be particularly useful for identifying efficient varieties at limiting N conditions.
- High leaf chlorophyll, as measured by high SPAD values, could be used as an indication of a variety having high grain N yield, and likely high NUE.
- The functional allele of *NAM-B1* has been preserved in many varieties from Denmark, Finland, Norway and Sweden.
- Varieties with the functional allele of *NAM-B1* did not have higher grain N concentrations, contrary to the expectation. These varieties did however have accelerated senescence and more ears. The effect of the gene in different controlled environments needs to be further investigated.

References

Ågren, G. I. (1985). Theory for growth of plants derived from the nitrogen productivity concept. Physiologia Plantarum 64(1), 17–28.

Åkerman, Å. (1951). Svensk växtförädling. Stockholm: Natur och Kultur.

Araus, J. L., Slafer, G. A., Reynolds, M. P. & Royo, C. (2002). Plant Breeding and Drought in C3 Cereals: What Should We Breed For? *Annals of Botany* 89(7), 925–940.

Asplund, L., Bergkvist, G., Leino, M. W., Westerbergh, A. & Weih, M. (2013). Swedish Spring Wheat Varieties with the Rare High Grain Protein Allele of *NAM-B1* Differ in Leaf Senescence and Grain Mineral Content. *PLoS ONE* 8(3), e59704.

Asplund, L., Hagenblad, J. & Leino, M. W. (2010). Re-evaluating the history of the wheat domestication gene *NAM-B1* using historical plant material. *Journal of Archaeological Science* 37(9), 2303–2307.

Austin, R. B., Bingham, J., Blackwell, R. D., Evans, L. T., Ford, M. A., Morgan, C. L. & Taylor, M. (1980). Genetic improvements in winter wheat yields since 1900 and associated physiological changes. *The Journal of Agricultural Science* 94(03), 675–689.

Avivi, L. (1978). High grain protein content in wild tetraploid wheat *Triticum dicoccoides* Korn., 1978. pp 372–380.

Avni, R., Zhao, R., Pearce, S., Jun, Y., Uauy, C., Tabbita, F., Fahima, T., Slade, A., Dubcovsky, J. & Distelfeld, A. (2014). Functional characterization of GPC-1 genes in hexaploid wheat. *Planta* 239(2), 313–324.

Le Bail, M., Jeuffroy, M.-H., Bouchard, C. & Barbottin, A. (2005). Is it possible to forecast the grain quality and yield of different varieties of winter wheat from Minolta SPAD meter measurements? *European Journal of Agronomy* 23(4), 379–391.

Balfourier, F., Roussel, V., Strelchenko, P., Exbrayat-Vinson, F., Sourdille, P., Boutet, G., Koenig, J., Ravel, C., Mitrofanova, O., Beckert, M. & Charmet, G. (2007). A worldwide bread wheat core collection arrayed in a 384-well plate. *Theoretical and Applied Genetics* 114(7), 1265–1275.

Baresel, J. P., Zimmermann, G. & Reents, H. J. (2008). Effects of genotype and environment on N uptake and N partition in organically grown winter wheat (*Triticum aestivum L.*) in Germany. *Euphytica* 163(3), 347–354.

Berendse, F. & Aerts, R. (1987). Nitrogen-Use-Efficiency: A Biologically Meaningful Definition? *Functional Ecology* 1(3), 293–296.

Bertholdsson, N.-O. & Kolodinska Brantestam, A. (2009). A century of Nordic barley breeding - Effects on early vigour root and shoot growth, straw length, harvest index and grain weight. *European Journal of Agronomy* 30(4), 266–274.

Bogard, M., Allard, V., Brancourt-Hulmel, M., Heumez, E., Machet, J.-M., Jeuffroy, M.-H., Gate, P., Martre, P. & Le Gouis, J. (2010). Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. *Journal of Experimental Botany* 61(15), 4303 –4312.

Borrell, A. K. & Hammer, G. L. (2000). Nitrogen Dynamics and the Physiological Basis of Stay-Green in Sorghum. *Crop Sci* 40(5), 1295–1307.

Borrell, A. K., Hammer, G. L. & Henzell, R. G. (2000). Does Maintaining Green Leaf Area in Sorghum Improve Yield under Drought? II. Dry Matter Production and Yield. *Crop Sci.* 40(4), 1037–1048.

Bouwman, A. F., Boumans, L. J. M. & Batjes, N. H. (2002). Emissions of N2O and NO from fertilized fields: Summary of available measurement data. *Global Biogeochemical Cycles* 16(4), 1058.

Brancourt-Hulmel, M., Heumez, E., Pluchard, P., Beghin, D., Depatureaux, C., Giraud, A. & Le Gouis, J. (2005). Indirect versus Direct Selection of Winter Wheat for Low-Input or High-Input Levels. *Crop Science.* 45(4), 1427–1431.

Brevis, J. C. & Dubcovsky, J. (2010). Effects of the Chromosome Region Including the *Gpc-B1* Locus on Wheat Grain and Protein Yield. *Crop Science* 50(1), 93–104.

Buchanan-Wollaston, V., Page, T., Harrison, E., Breeze, E., Lim, P. O., Nam, H. G., Lin, J.-F., Wu, S.-H., Swidzinski, J., Ishizaki, K. & Leaver, C. J. (2005). Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in Arabidopsis. *The Plant Journal* 42(4), 567–585.

Calderini, D. F., Torres-León, S. & Slafer, G. A. (1995). Consequences of Wheat Breeding on Nitrogen and Phosphorus Yield, Grain Nitrogen and Phosphorus Concentration and Associated Traits. *Annals of Botany* 76(3), 315–322.

Campbell, K. G. (1997). Spelt: agronomy, genetics, and breeding. *Plant Breeding Reviews* 15, 187–214.

Cantrell, R. G. & Joppa, L. R. (1991). Genetic Analysis of Quantitative Traits in Wild Emmer (*Triticum turgidum* L. var. *dicoccoides*). *Crop Science* 31(3), 645–649.

Cantu, D., Pearce, S., Distelfeld, A., Christiansen, M., Uauy, C., Akhunov, E., Fahima, T. & Dubcovsky, J. (2011). Effect of the down-regulation of the high *Grain Protein Content* (*GPC*) genes on the wheat transcriptome during monocarpic senescence. *BMC Genomics* 12(1), 492.

Carter, A. H., Santra, D. K. & Kidwell, K. K. (2012). Assessment of the effects of the *Gpc-B1* allele on senescence rate, grain protein concentration and mineral content in hard red spring wheat (*Triticum aestivum* L.) from the Pacific Northwest Region of the USA. *Plant Breeding* 131(1), 62–68.

Ceccarelli, S. (1996). Adaptation to low/high input cultivation. *Euphytica* 92(1), 203–214.

Chapin, F. S. (1980). The Mineral Nutrition of Wild Plants. *Annual Review of Ecology and Systematics* 11(1), 233–260.

Chee, P., Elias, E., Anderson, J. & Kianian, S. (2001). Evaluation of a high grain protein QTL from *Triticum turgidum* L. var. *dicoccoides* in an adapted durum wheat background. *Crop Science* 41(2), 295–301.

Cunniff, J., Wilkinson, S., Charles, M., Jones, G., Rees, M. & Osborne, C. P. (2014). Functional Traits Differ between Cereal Crop Progenitors and Other Wild Grasses Gathered in the Neolithic Fertile Crescent. *PLoS ONE* 9(1), e87586.

Distelfeld, A., Avni, R. & Fischer, A. M. (2014). Senescence, nutrient remobilization, and yield in wheat and barley. *Journal of Experimental Botany* [online]. Available from: http://jxb.oxfordjournals.org/content/early/2014/01/24/jxb.ert477.abstract.

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(4), 753–763.

Dubcovsky, J. & Dvorak, J. (2007). Genome Plasticity a Key Factor in the Success of Polyploid Wheat Under Domestication. *Science* 316(5833), 1862–1866.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B. & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* 10(12), 1135–1142.

Evans, J. (1989). Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* 78(1), 9–19.

Fageria, N. K., Baligar, V. C. & Li, Y. C. (2008). The role of nutrient efficient plants in improving crop yields in the twenty first century. *Journal of Plant Nutrition* 31(6), 1121–1157.

FAOSTAT. [online]. Available from: http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E. [Accessed 2014-03-03].

Fischer, R. A. (2008). The importance of grain or kernel number in wheat: A reply to Sinclair and Jamieson. *Field Crops Research* 105(1–2), 15 – 21.

Fischer, R. A., Howe, G. N. & Ibrahim, Z. (1993). Irrigated spring wheat and timing and amount of nitrogen fertilizer. I. Grain yield and protein content. *Field Crops Research* 33(1–2), 37–56.

Flink, M., Pettersson, R. & Andrén, O. (1995). Growth dynamics of winter wheat in the field with daily fertilization and irrigation. *Journal of Agronomy and Crop Science* 174(4), 239–252.

Gaju, O., Allard, V., Martre, P., Snape, J. W., Heumez, E., LeGouis, J., Moreau, D., Bogard, M., Griffiths, S., Orford, S., Hubbart, S. & Foulkes, M. J. (2011). Identification of traits to improve the nitrogen-use efficiency of wheat genotypes. *Field Crops Research* 123(2), 139–152.

Guarda, G., Padovan, S. & Delogu, G. (2004). Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. *European Journal of Agronomy* 21(2), 181–192.

Guttieri, M., Stein, R. & Waters, B. (2013). Nutrient partitioning and grain yield of *TaNAM*-RNAi wheat under abiotic stress. *Plant and Soil* 371(1-2), 573–591.

Hagenblad, J., Asplund, L., Balfourier, F., Ravel, C. & Leino, M. (2012). Strong presence of the high grain protein content allele of *NAM-B1* in Fennoscandian wheat. *Theoretical and Applied Genetics* 125(8), 1677–1686.

Hay, R. & Porter, J. (2006). *The physiology of crop yield*. Oxford, UK Ames, Iowa: Oxford, UK Blackwell Publishing Ames, Iowa.

Herrera, J. M., Noulas, C., Feil, B., Stamp, P. & Liedgens, M. (2013). Nitrogen and genotype effects on root growth and root survivorship of spring wheat. *Journal of Plant Nutrition and Soil Science* 176(4), 561–571.

Van Herwaarden, A. F., Farquhar, G. D., Angus, J. F., Richard, R. A. & Howe, G. N. (1998). ”Haying-off”, the negative grain yield response of dryland wheat to nitrogen fertiliser. I. Biomass, grain yield, and water use. *Australian Journal of Agricultural Research* 49(7), 1067–1081.

Holford, I., Doyle, A. & Leckie, C. (1992). Nitrogen response characteristics of wheat protein in relation to yield responses and their interactions with phosphorus. *Australian Journal of Agricultural Research* 43(5), 969–986.

Howarth, J. R., Parmar, S., Jones, J., Shepherd, C. E., Corol, D.-I., Galster, A. M., Hawkins, N. D., Miller, S. J., Baker, J. M., Verrier, P. J., Ward, J. L., Beale, M. H., Barraclough, P. B. & Hawkesford, M. J. (2008). Co-ordinated expression of amino acid metabolism in response to N and S deficiency during wheat grain filling. *Journal of Experimental Botany* 59(13), 3675–3689.

Ingestad, T. (1979). Nitrogen Stress in Birch Seedlings. *Physiologia Plantarum* 45(1), 149–157.

Joppa, L. R., Du, C., Hart, G. E. & Hareland, G. A. (1997). Mapping Gene(s) for Grain Protein in Tetraploid Wheat (*Triticum turgidum* L.) Using a Population of Recombinant Inbred Chromosome Lines. *Crop Science* 37(5), 1586–1589.

Khush, G. S. (1999). Green revolution: preparing for the 21st century. *Genome* 42(4), 646–655.

Kibite, S. & Evans, L. E. (1984). Causes of negative correlations between grain yield and grain protein concentration in common wheat. *Euphytica* 33(3), 801–810.

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

King, J., Gay, A., Sylvester-Bradley, R., Bingham, I., Foulkes, J., Gregory, P. & Robinson, D. (2003). Modelling Cereal Root Systems for Water and Nitrogen Capture: Towards an Economic Optimum. *Annals of Botany* 91(3), 383–390.

Kirby, E. J. M. (1988). Analysis of leaf, stem and ear growth in wheat from terminal spikelet stage to anthesis. *Field Crops Research* 18(2–3), 127 – 140.

Kumar, J., Jaiswal, V., Kumar, A., Kumar, N., Mir, R. R., Kumar, S., Dhariwal, R., Tyagi, S., Khandelwal, M., Prabhu, K. V., Prasad, R., Balyan, H. S. & Gupta, P. K. (2011). Introgression of a major gene for high grain protein content in some Indian bread wheat cultivars. *Field Crops Research* 123(3), 226–233.

Lacerenza, J. A., Parrott, D. L. & Fischer, A. M. (2010). A major grain protein content locus on barley (*Hordeum vulgare* L.) chromosome 6 influences flowering time and sequential leaf senescence. *Journal of Experimental Botany* 61(11), 3137–3149.

Larsson, S., Hagman, J. & Kent, D. (2012). *Stråsäd, trindsäd, oljeväxter, potatis : sortval 2012*. Uppsala: Institutionen för växtproduktionsekologi, SLU. ISBN 978-91-576-9066-1.

Leino, M. W., Hagenblad, J., Edqvist, J. & Strese, E.-M. K. (2009). DNA preservation and utility of a historic seed collection. *Seed Science Research* 19(03), 125–135.

Liao, M., Fillery, I. R. P. & Palta, J. A. (2004). Early vigorous growth is a major factor influencing nitrogen uptake in wheat. *Functional Plant Biology* 31(2), 121–129.

Liao, M., Palta, J. A. & Fillery, I. R. P. (2006). Root characteristics of vigorous wheat improve early nitrogen uptake. *Australian Journal of Agricultural Research* 57(10), 1097–1107.

Lim, P. O., Kim, H. J. & Gil Nam, H. (2007). Leaf senescence. *Annual Review of Plant Biology* 58, 115 – 136.

Löschenberger, F., Fleck, A., Grausgruber, H., Hetzendorfer, H., Hof, G., Lafferty, J., Marn, M., Neumayer, A., Pfaffinger, G. & Birschitzky, J. (2008). Breeding for organic agriculture: the example of winter wheat in Austria. *Euphytica* 163(3), 469–480.

Maathuis, F. J. (2009). Physiological functions of mineral macronutrients. *Current Opinion in Plant Biology* 12(3), 250–258.

Malhi, S. S., Johnston, A. M., Schoenau, J. J., Wang, Z. L. & Vera, C. L. (2006). Seasonal biomass accumulation and nutrient uptake of wheat, barley and oat on a Black Chernozem Soil in Saskatchewan. *Canadian Journal of Plant Science* 86(4), 1005–1014.

Maydup, M. L., Graciano, C., Guiamet, J. J. & Tambussi, E. A. (2012). Analysis of early vigour in twenty modern cultivars of bread wheat (*Triticum aestivum* L.). *Crop and Pasture Science* 63(10), 987–996.

Mesfin, A., Frohberg, R. C. & Anderson, J. A. (1999). RFLP Markers Associated With High Grain Protein From *Triticum Turgidum* L. Var. *Dicoccoides* Introgressed Into Hard Red Spring Wheat. *Crop Science* 39(2), 508–513.

Moll, R. H., Kamprath, E. J. & Jackson, W. A. (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen-utilization. *Agronomy Journal* 74(3), 562–564.

Muurinen, S., Slafer, G. A. & Peltonen-Sainio, P. (2006). Breeding effects on nitrogen use efficiency of spring cereals under northern conditions. *Crop Science* 46(2), 561–568.

Noodén, L. D. (1988). *Senescence and aging in plants*. Access Online via Elsevier. ISBN 0323145957.

Näsholm, T., Huss-Danell, K. & Högberg, P. (2001). Uptake of glycine by field grown wheat. *New Phytologist* 150(1), 59–63.

Olsen, A. N., Ernst, H. A., Leggio, L. L. & Skriver, K. (2005). NAC transcription factors: structurally distinct, functionally diverse. *Trends in Plant Science* 10(2), 79 – 87.

Ortiz, R., Madsen, S. & Andersen, S. B. (1998). Diversity in Nordic spring wheat cultivars (1901–93). *Acta Agriculturae Scandinavica, Section B - Plant Soil Science* 48(4), 229 – 238.

Ozturk, A. & Aydin, F. (2004). Effect of Water Stress at Various Growth Stages on Some Quality Characteristics of Winter Wheat. *Journal of Agronomy and Crop Science* 190(2), 93–99.

Ozturk, A., Caglar, O. & Bulut, S. (2006). Growth and Yield Response of Facultative Wheat to Winter Sowing, Freezing Sowing and Spring Sowing at Different Seeding Rates. *Journal of Agronomy and Crop Science* 192(1), 10–16.

Parrott, D. L., Downs, E. P. & Fischer, A. M. (2012). Control of barley (*Hordeum vulgare* L.) development and senescence by the interaction between a chromosome six grain protein content locus, day length, and vernalization. *Journal of Experimental Botany* 63(3), 1329 – 1339.

Peltonen, J., Virtanen, A. & Haggrèn, E. (1995). Using a Chlorophyll Meter to Optimize Nitrogen Fertilizer Application for Intensively-Managed Small-Grain Cereals. *Journal of Agronomy and Crop Science* 174(5), 309–318.

Peltonen-Sainio, P., Jahuhianinen, L. & Hakala, K. (2011). Crop responses to temperature and precipitation according to long-term multi-location trials at high-latitude conditions. *The Journal of Agricultural Science* 149(01), 49–62.

Peltonen-Sainio, P., Jauhiainen, L. & Laurila, I. P. (2009). Cereal yield trends in northern European conditions: Changes in yield potential and its realisation. *Field Crops Research* 110(1), 85 – 90.

Peltonen-Sainio, P., Jauhiainen, L. & Nissilä, E. (2012). Improving cereal protein yields for high latitude conditions. *European Journal of Agronomy* 39, 1–8.

Peltonen-Sainio, P., Kangas, A., Salo, Y. & Jauhiainen, L. (2007). Grain number dominates grain weight in temperate cereal yield determination: Evidence based on 30 years of multi-location trials. *Field Crops Research* 100(2–3), 179–188.

Pieterse, C. M. J., Leon-Reyes, A., Van der Ent, S. & Van Wees, S. C. M. (2009). Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology* 5(5), 308–316.

Porter, J. R. & Gawith, M. (1999). Temperatures and the growth and development of wheat: a review. *European Journal of Agronomy* 10(1), 23–36.

Rajala, A., Hakala, K., Mäkelä, P., Muurinen, S. & Peltonen-Sainio, P. (2009). Spring wheat response to timing of water deficit through sink and grain filling capacity. *Field Crops Research* 114(2), 263–271.

Rebetzke, G., Botwright, T., Moore, C., Richards, R. & Condon, A. (2004). Genotypic variation in specific leaf area for genetic improvement of early vigour in wheat. *Field Crops Research* 88(2–3), 179–189.

Reich, P. B., Walters, M. B., Tjoelker, M. G., Vanderklein, D. & Buschena, C. (1998). Photosynthesis and Respiration Rates Depend on Leaf and Root Morphology and Nitrogen Concentration in Nine Boreal Tree Species Differing in Relative Growth Rate. *Functional Ecology* 12(3), 395–405.

Reynolds, M., Foulkes, M. J., Slafer, G. A., Berry, P., Parry, M. A. J., Snape, J. W. & Angus, W. J. (2009). Raising yield potential in wheat. *Journal of Experimental Botany* 60(7), 1899–1918.

Richards, R. A. & Lukacs, Z. (2002). Seedling vigour in wheat - sources of variation for genetic and agronomic improvement. *Australian Journal of Agricultural Research* 53(1), 41–50.

Rüegger, A., Winzeler, M. & Winzeler, H. (1993). The Influence of Different Nitrogen Levels and Seeding Rates on the Dry Matter Production and Nitrogen Uptake of Spelt (*Triticum spelta* L.) and Wheat (*Triticum aestivum* L.) under Field Conditions. *Journal of Agronomy and Crop Science* 171(2), 124–132.

Schulte auf'm Erley, G., Begum, N., Worku, M., Bänziger, M. & Horst, W. J. (2007). Leaf senescence induced by nitrogen deficiency as indicator of genotypic differences in nitrogen efficiency in tropical maize. *Journal of Plant Nutrition and Soil Science* 170(1), 106–114.

Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany* 60(6), 1537–1553.

Siddiqi, M. & Glass, A. (1981). Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. *Journal of Plant Nutrition* 4(3), 289–302.

Sinclair, T. R. (1998). Historical Changes in Harvest Index and Crop Nitrogen Accumulation. *Crop Science* 38(3), 638–643.

Sinclair, T. R. & Jamieson, P. D. (2006). Grain number, wheat yield, and bottling beer: An analysis. *Field Crops Research* 98(1), 60–67.

Sinclair, T. R., Purcell, L. C. & Sneller, C. H. (2004). Crop transformation and the challenge to increase yield potential. *Trends in Plant Science* 9(2), 70 – 75.

Slafer, G. A., Abeledo, L. G., Miralles, D. J., Gonzalez, F. G. & Whitechurch, E. M. (2001). Photoperiod sensitivity during stem elongation as an avenue to raise potential yield in wheat. *Euphytica* 119(1-2), 191–197.

Slafer, G. & Andrade, F. (1991). Changes in physiological attributes of the dry matter economy of bread wheat (*Triticum aestivum*) through genetic improvement of grain yield potential at different regions of the world. *Euphytica* 58(1), 37–49.

Smil, V. (1999). Nitrogen in crop production: An account of global flows. *Global Biogeochemical Cycles* 13(2), 647–662.

Spaner, D., Todd, A. G., Navabi, A., McKenzie, D. B. & Goonewardene, L. A. (2005). Can Leaf Chlorophyll Measures at Differing Growth Stages be used as an Indicator of Winter Wheat and Spring Barley Nitrogen Requirements in Eastern Canada? *Journal of Agronomy and Crop Science* 191(5), 393–399.

Steiger, D. K., Elias, E. M. & Cantrell, R. G. (1996). Evaluation of Lines Derived from Wild Emmer Chromosome Substitutions: I. Quality Traits. *Crop Science* 36(2), 223–227.

Suding, K. N., Collins, S. L., Gough, L., Clark, C., Cleland, E. E., Gross, K. L., Milchunas, D. G. & Pennings, S. (2005). Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the United States of America* 102(12), 4387–4392.

Swedish Commission on Climate and Vulnerability (2007). *Sweden facing climate change : threats and opportunities : final report*. Stockholm: Fritze. (SOU; 2007:60). ISBN 978-91-38-22850-0.

Sylvester-Bradley, R. & Kindred, D. R. (2009). Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. *Journal of Experimental Botany* 60(7), 1939–1951.

Tabbita, F., Lewis, S., Vouilloz, J. P., Ortega, M. A., Kade, M., Abbate, P. E. & Barneix, A. J. (2013). Effects of the *Gpc-B1* locus on high grain protein content introgressed into Argentinean wheat germplasm. *Plant Breeding* 132(1), 48–52.

Taiz, L. & Zeiger, E. (2006). *Plant physiology*. Sunderland, Massachusetts: Sinauer Associates.

Thomas, H. & Howarth, C. J. (2000). Five ways to stay green. *Journal of Experimental Botany* 51(suppl 1), 329–337.

Thorup-Kristensen, K., Salmeron Cortasa, M. & Loges, R. (2009). Winter wheat roots grow twice as deep as spring wheat roots, is this important for N uptake and N leaching losses? *Plant and Soil* 322(1-2), 101–114.

Tottman, D. (1987). The decimal code for the growth-stages of cereals, with illustrations. *Annals of Applied Biology* 110(2), 441–454.

Uauy, C., Brevis, J. C. & Dubcovsky, J. (2006a). The high grain protein content gene *Gpc-B1* accelerates senescence and has pleiotropic effects on protein content in wheat. *Journal of Experimental Botany* 57(11), 2785 –2794.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A. & Dubcovsky, J. (2006b). A *NAC* gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314, 1298 – 1301.

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S. C., Prasad, S. V. S., Rebetzke, G. J., Kirkegaard, J. A., Christopher, J. & Watt, M. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany* 63(9), 3485–3498.

Waters, B. M., Uauy, C., Dubcovsky, J. & Grusak, M. A. (2009). Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *Journal of Experimental Botany* 60(15), 4263–4274.

Watt, M., Kirkegaard, J. A. & Rebetzke, G. J. (2005). A wheat genotype developed for rapid leaf growth copes well with the physical and biological constraints of unploughed soil. *Functional Plant Biology* 32(8), 695–706.

Weih, M., Asplund, L. & Bergkvist, G. (2011). Assessment of nutrient use in annual and perennial crops: A functional concept for analyzing nitrogen use efficiency. *Plant and Soil* 339(1), 513–520.

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. (2007). Let the concept of trait be functional! *Oikos* 116(5), 882–892.

Vitousek, P. (1982). Nutrient Cycling and Nutrient Use Efficiency. *The American Naturalist* 119(4), 553–572.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H. & Tilman, D. G. (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7(3), 737–750.

Zadoks, J. C., Chang, T. T. & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research* 14(6), 415–421.

Zohary, D., Hopf, M. & Weiss, E. (2012). *Domestication of plants in the Old World : the origin and spread of domesticated plants in south-west Asia, Europe, and the Mediterranean Basin.* 4th ed. Oxford: Oxford University Press. ISBN 9780199549061

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