Screening of \( HvNAM-B1 \) polymorphism, grain nutrient content and seed size in 80 Scandinavian barley cultivars

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Titel:

Screening of HvNAM-B1 polymorphism, grain nutrient content and seed size in 80 Scandinavian barley cultivars

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Sammanfattning

Abstract: Micronutrient malnutrition is a leading worldwide health problem that affects billions of people particularly in the developing countries resulting in serious health conditions. The domestication of crops produced high yield and larger seed size but with a reduction in nutritious quality. The locus NAM or Gpc-1 affects both the seed size and nutrient content in wheat and barley. A non-functional allele of the gene increases the seed size but at the expense of protein and micronutrient content. However, this gene was found to be lost in wheat during the early domestication resulting in lower nutrient content. Therefore, the selection for high yield has lead to lower grain nutrients. Our aim of the present study is to investigate when the selection for yield occured in barley and to check the existence of the wild type allele in the 19th century of landrace barley crops. In addition, to analyse the barley grain concentration of protein, iron and zinc among the landrace and cultivars from various time periods of northern Europe. The grain nutrient concentration of Nitrogen, Iron and Zinc did not show significant difference among the investigated 80 Scandinavian barley accessions. The grain nutrient concentration did not correlate with the seed size and chlorophyll content. The polymorphism was not observed among the allelic diversity of HvNAM-1 gene indicating that the NAM-B1 gene still prevails in the 19th century barley cultivars.

Nyckelord

Keyword: Micronutrient Malnutrition, NAM-B1, Yield, Seed size, Northern Europe
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Abstract

Micronutrient malnutrition is a leading worldwide health problem that affects billions of people particularly in the developing countries resulting in serious health conditions. The domestication of crops produced high yield and larger seed size but with a reduction in nutritious quality. The locus NAM or Gpc-1 affects both the seed size and nutrient content in wheat and barley. A non-functional allele of the gene increases the seed size but at the expense of protein and micronutrient content. However, this gene was found to be lost in wheat during the early domestication resulting in lower nutrient content. Therefore, the selection for high yield has lead to lower grain nutrients. Our aim of the present study is to investigate when the selection for yield occurred in barley and to check the existence of the wild type allele in the 19th century of landrace barley crops. In addition, to analyse the barley grain concentration of protein, iron and zinc among the landrace and cultivars from various time periods of northern Europe. The grain nutrient concentration of Nitrogen, Iron and Zinc did not show significant difference among the investigated 80 Scandinavian barley accessions. The grain nutrient concentration did not correlate with the seed size and chlorophyll content. The polymorphism was not observed among the allelic diversity of HvNAM-1 gene indicating that the NAM-B1 gene still prevails in the 19th century barley cultivars.

Keywords: Micronutrient malnutrition, NAM-B1, Yield, seed size, Northern Europe

Abbreviations:

NAM- No Apical Meristem, ANOVA- Analysis of Variance, HvNAM-1- Hordeum vulgare, No Apical Meristem, GPC-1- Grain Protein Content-1, CAPS- Cleaved Amplified Polymorphic Sequence, TBE- Tris-Borate-EDTA, ATAF- Arabidopsis thaliana transcription Factor, CUC2- Cup-shaped Cotyledon, a NAC protein, STM- Shoot Meristemless, NordGen- the Nordic Genetic Resource Center, SNP- Single Nucleotide Polymorphism

1. Introduction

The improvement of agriculture is dependent on the domestication of wheat, one of the earliest crop species and has been of major significance for human consumption (Dubcovsky and Dvorak, 2007). In recent years, micronutrient malnutrition is one of the most outstanding problems affecting billions of people, especially in the developing countries leading to poor health conditions. The development of micronutrient enriched cereals using genetic tools could be an effective approach to improve the quality of the food and sustainably reduce malnutrition worldwide. However, this requires a complete understanding of the mechanisms controlling accumulation and remobilization of nutrients in the harvest products. The locus NAM-1 or grain protein content-1 (GPC -1) affects the seed size and nutrient content and also has a role in senescence in wheat and barley (Distelfeld et al., 2007). The non-functional allele of this gene increases the seed size but at the expense of protein and micronutrient content (Distelfeld et al., 2007).
1.1 Barley

Cereals are an important source of micronutrient minerals essential for human consumption (Zhao et al., 2009) and barley is widely grown as a commercial crop in about 100 countries (Mahdi et al., 2008). Cereal products provide 44% of the daily intake of Fe (15% from bread), 27% of Mg (13% from bread), 25% of Zn (11% from bread) and 31% of Cu (14% from bread)(Fan et al., 2008). Barley holds the fourth position in the overall cereal production worldwide, accounting nearly 30% of the total worldwide and is highly enriched with chromium and fibers (Mahdi et al., 2008). About 85% of the world barley production is assigned for animal consumption, while the remaining is used in malt production, seed production, chemical industry and brewing industry (Fischbeck, 2002). Improvement of semi-dwarf crop varieties has significantly increased the world food production because of their good resistance, higher yield and better utilization of environment. Since 1930, the semi-dwarf barley cultivars has increased the world grain production in response to fertilizers (Zhang et al., 2006).

The word “Landrace” is defined as a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming system (Villa et al., 2005). The widely accepted definition for landrace is “a variety of crop with high capacity to tolerate abiotic and biotic stress results in high yielding stability and an intermediate yield level even under low input agricultural system. The two different types of landraces are autochthonous and allochthonous. The landraces which are cultivated in a specific region for more than a century is called “autochthonous”. The autochthonous landrace in a region is introduced and domesticated in another region is called “allochthonous” (Zeven et al., 1998). “Modern cultivars” can be defined as the crops improved by breeding techniques which are grown under optimal conditions for high yield (Villa et al., 2005 and Zeven et al., 1998). The landraces are specifically used for modern crop improvement to maintain the genetic integrity, to avoid genetic erosion and for the conservation of the different crop genotypes (Joshi et al., 2004 and Villa et al., 2005). For the last 100 years, barley has undergone modern plant breeding which resulted in cultivars with high yield, better quality and improved pest resistance. In Europe, most of the landrace crops were replaced by modern cultivars in the 1920s (Fischbeck, 2003).

1.2 Micronutrient deficiency:

Micronutrient malnutrition is a world leading serious health problem prevailing in the developing countries, particularly among women and children (World Health Organisation, 2002). It has been estimated that Iron and Zinc deficiencies affected one-third of the world’s population (Zhao et al., 2009). The selection for high yielding modern crops in wheat and rice has increased in South Asia and this might have caused the rise in micronutrient deficiency (Welch and Graham, 2002). However, whether the increased grain yield has resulted in lower mineral content and the evidence for this still remains controversial (Garvin et al., 2006; Graham et al., 1999; McGrath, 1985; Oury et al., 2006).
1.3 Grain protein content (GPC)

GPC is an important factor in the quality of cereals and also play a significant role in malting and brewing process (Jamar et al., 2010). In barley, the proteins are closely associated with the malting quality by their various roles in enzyme activities (Clancy et al., 2003) and in yeast nutrition, haze formation in beer (See et al., 2002). The previous studies reported that the Gpc-B1 affects the grain mineral concentration and senescence (Distelfeld et al., 2007). Grain protein content possess negative relationship with grain yield in wheat lines as experimented by (Zhao et al., 2009), indicating that the plant breeding aiming for high grain yield has resulted in a lower quantity of grain protein content.

1.4 NAC

The acronym NAC is basically derived from the names of three homologous genes namely, NAM (No Apical Meristem), ATAF1,2 (Arabidopsis thaliana transcription factor) and CUC2 (cup- shaped cotyledon) (Ernst et al., 2004). The NAC proteins share a similar N-terminal domain called the NAC domain, which is a DNA binding domain. In the C-terminal most NAC proteins carry a transcription regulatory domain. The NAC domain has only been identified in plants and the NAC proteins make up one of the largest families of plant-specific transcription factor. The NAM was the first gene to be characterized in Arabidopsis thaliana, followed by CUC2 gene (Cup-Shaped Cotyledon 2). The CUC1 gene, a NAC encoded domain protein having sequence similar to CUC2 and involved in the establishment of cotyledon boundary and shoot apical meristem (Olsen et al., 2005). From the previous studies, it was found that there is an interaction between CUC genes and STM (Shoot Mersitemless) gene (Olsen et al., 2005). The STM and CLAVATA genes helps to regulate shoot meristem development but with opposite function. CLAVATA helps to promote the differentiation of cells in the shoot and floral meristem, while STM restricts the cell differentiation in the shoot and floral meristem (Clark et al., 1996).

The NAC proteins participate in various plant processes such as in the development of shoot apical meristem, floral organs and in formation of lateral roots (Souer et al., 1996; Aida et al., 1997; Xie et al., 2000). Thus, the NAC proteins are considered to be important proteins in plant development and biology. However, the x-ray crystal structure of NAC domain was identified to understand the function of NAC proteins at the molecular level by using X-ray Crystallography (Ernst et al., 1996). The crystallographic structural studies indicates that the NAC domain helps in mediating the dimerization of NAC proteins through a interaction with salt bridge located between the residues and contains a DNA binding region, where the NAC dimer protrude out into positively charged residues (Ernst et al., 1996).

1.5 No Apical Meristem (NAM)

The NAM-B1 is a wild type allele in wheat that encodes a transcription factor of the NAC family. It accelerates the maturity and helps in increasing the mobilization of nutrients from leaves to developing grains. The modern wheat crops contain a non-functional NAM-B1 allele that delays the maturity and results in higher yields but also leads to a decrease in the protein, iron and zinc contents in the grains. Therefore, the selection for yield leads to a reduction in nutritious quality (Uauy et al., 2006a).
The single gene Gpc-B1 is an allele of NAM-B1 and have a frame shift mutation which, if translated, would result in a protein without the NAC domain (Uauy et al., 2006a) This gene has multiple pleiotrophic effects, which are special characteristics of NAC transcription factors. The effects were found to be essential in developmental processes, auxin signaling, defense mechanism, abiotic stress response and leaf senescence (Uauy et al., 2006b). According to Uauy et al., (2006a), the function of NAM-B1 gene was thought to be lost during the domestication of wheat. This hypothesis was tested by Asplund et al., (2010) and they managed to find the functional allele in several 19th century cultivars. The genes other than NAM-B1 are also important for GPC variation and the NAC proteins are encoded by multigene families (Jamar et al., 2010).

2. Objectives of this study

The objectives of the present study were to explore the diversity of the HvNAM-1 gene sequence and the grain protein, zinc, and iron content in Scandinavian landraces and cultivars from four different time periods

3. Materials and Methods

3.1 Plant material

Eighty-two different accessions of barley from Nordic countries were obtained from NordGen (the Nordic Genetic Resource Center). The plants consisted of four groups: Landraces, old cultivars (1890-1940), cultivars (1941-1970) and modern cultivars (1971- present). Furthermore, accessions from the countries Norway, Sweden, Denmark and Finland were equally represented within each group. Two barley varieties, ‘Karl’ and ‘Lewis’, were received from the Germplasm Research facility of the Small Grains Collection, US (United States) and were used as controls. The ‘Karl’ control with accession number Clho 15487 has low GPC and the ‘Lewis’ control with accession number Clho 15856 has high GPC. ‘Karl’ and ‘Lewis’ are polymorphic in two SNPs (Single Nucleotide Polymorphism) positioned in NAM (Distelfeld et al., 2008).

3.2 Plant growth study

The barley plants were cultivated in a greenhouse for three months at Linköping University. Three replicates of each accession were grown and the plants were well watered, supplied with fertilizers (Algomin, Linköping, Sweden) containing nutrients like N (4%), P (5%), K (11%) and S (8.6%) initially (after two weeks) to enrich their growth. No additional fertilizers were given during the growth period. The maturation time was recorded after the appearance of the first spike in each plant. Twenty days after spike appearance, the chlorophyll content in the leaf (measured in grams) was recorded by a SPAD instrument (Minolta, Mumbai, India) for all the three plant replicates during maturation stage. The grains were harvested manually after three months of maturity. After 94 days, the height of the plant from soil surface to the top of primary spike was measured for each plant. Three spikes from each plant (or less if more spikes were not available) were cut and placed in a paper bag to measure the weight of fresh grain. Following, the grains were dried in a heating cupboard for 48h at 60°C and the dry weight was measured. The dried grains were grounded and threshed thoroughly to remove all chaff and were counted to obtain 30 grains to measure its seed size. The grain samples were analysed for
mineral concentration. Analyses of Nitrogen (Leco Corp, Lakeview Avenue, United States), and Iron and Zinc content was measured using Inductively Coupled Plasma atomic emission spectrometry at Agrilab AB in Uppsala.

3.3 DNA Extraction

The DNA extraction was performed using Qiagen DNAeasy Plant Mini Kit (Qiagen, Germany). Leaf tissue samples from the various different barley lines were collected and crushed thoroughly in the eppendorf tubes and the isolation was carried out according to the protocol in the kit.

3.4 PCR amplification

The PCRs were run in a 20µl reaction volume consisting of 1U of 5U/µl Dream Taq DNA polymerase enzyme (Fermentas, Helsingborg, Sweden), 1x Dream Taq reaction buffer (with 20mM Mg), 0.2 mM dNTP, 0.2 µM forward primer, 0.2 µM reverse primer and 1µl DNA template. The amplification was run at 94°C for 2min 30s in the initial denaturation step followed by 35cycles of 94°C for 30s, 58°C for 30s, and 72°C for 45s and a final extension step of 72°C for 7min.

3.5 CAPS (cleaved amplified polymorphic sequence) analysis

The CAPS are PCR markers carrying one single nucleotide polymorphism (SNP). The genetic loci uhb-6 and uhb-7 of HvNAM-1 were amplified using specific primers that were developed previously (Distelfeld et al., 2008). For CAPS analysis, the PCR products were digested with 1U Mwol (Fermentas) of in 20µl reaction volume for uhb6 and 1U Taal(Fermentas) for uhb7. The PCR products were analysed by gel electrophoresis in 1% agarose prepared in 1XTBE. All gels contained SybrSafe (Invitrogen) for the detection of the DNA in UV-light. Finally, the results were viewed on the Gel documentation system (BioDoc Imaging system, UVP, Cambridge, UK).

3.6 Data Analysis

Statistical analysis was performed for the obtained data using a General Linear Model-Univariate method and graphs were represented in the form of Error bars in the software SPSS Statistics. Correlation and regression analysis were performed for the 82 barley lines.

4. Results

4.1 PCR

Two cleavage amplified polymorphic sequence (CAPS) markers located to the barley HvNAM-1 gene were developed previously (Distelfeld et al.) Here we have these two markers, uhb6 and uhb7, to screen for genetic variation in the HvNAM-1 gene in 82 barley accessions representing Scandinavian landraces and cultivars from four different time periods. The barley cultivars Karl, which is a six-rowed malt barley that produces consistently lower GPC than other barley cultivars (Wesenberg et al., 1976), and Lewis which is a two-rowed cultivar with average GPC (Hockett et al., 1985) were included as controls. The PCR amplification of the HvNAM-1 gene
performed for the eighty two different varieties of barley lines was successful. The amplification of uhb6 resulted in a 469bp fragment (Fig.1A) and amplification of uhb7 yielded a 301bp fragment (Fig.1B). Thus, PCR products of the expected size were obtained from all samples and for both markers.

Fig 1. Gel images showing the DNA fragments Corresponding to the CAPS markers uhb6 and uhb7. (A) uhb6 primer combination produced a 469-bp fragment; (B) uhb7 primer combination produced a 301-bp fragment; M = DNA ladder mix, O'GeneRuler™(100bp) (Fermentas), l= cultivar before 1890, 2= cultivar from 1890-1940, 3= cultivar from 1941-1970, 4= cultivar from 1971-present, K = Karl(low GPC control), L = Lewis(high GPC control)

4.2 Analysis of allelic diversity of HvNAM-1 gene

The two SNPs (uhb6 and uhb7) detected between the Karl and Lewis HvNAM-1 alleles represent substitutions in two amino acid. The first SNP, uhb6, is located within the third NAC domain and results in the substitution of a Proline (Karl allele) to an Alanine (Lewis allele) at position 102 of HvNAM-1. The uhb7 SNP is located outside the NAC domain (position 357), in the C-terminal end of HvNAM-1, and results in an Alanine in the Lewis allele and a Threonine in the Karl allele of HvNAM-1(Distelfeld et al., 2008). Based on the amino acid combinations at these two positions, the Karl allele was designated ‘PT’ and Lewis allele ‘AA’(Distelfeld et al., 2008). The amplified uhb6 product from Karl produced an uncut 469-bp after digestion with Mwol while a 385-bp fragment was obtained from Lewis (fig. 2A). Similarly, the digestion of the uhb-7 product (301-bp) with restriction enzyme Taal revealed 164+137bp fragments in Karl and a 301bp uncut fragment in Lewis (fig. 2B). The controls Karl and Lewis were included with the
eighty samples to analyse the polymorphism of the allelic diversity of *Hv-NAM-1* gene among the four types of cultivars. In fig. 2C, the obtained cleaved fragments in negative control. Our present results show that among the four different types of barley accessions, the Lewis allele “AA” was observed in all the cultivars. The Karl allele “PT” was not observed in any cultivars suggesting lack of polymorphism (Appendix 1).

![Gel electrophoresis images showing no polymorphism among the different barley cultivars using CAPS markers](image)

**Fig 2.** Gel electrophoresis images showing no polymorphism among the different barley cultivars using CAPS markers (A) uhb6 (B) uhb7 showing strong bands in the four samples and controls(karl and lewis) (C) uhb7 produced weak fragments in the control allele(karl); 1= cultivar before 1890, 2= cultivar from 1890-1940, 3= cultivar from 1941-1970, 4= cultivar from 1971-present, *M = GeneRuler™, Low range DNA ladder(25bp), K = Karl(low GPC control), L = Lewis(high GPC control)*

### 4.3 Comparison of growth parameters among the cultivars

The barley lines were classified into four groups based on the different time periods and each group contains twenty different barley accessions (Appendix 1). chlorophyll content, plant height
and seed size were measured for the different barley varieties and compared among the four different groups using ANOVA. The results show a decreasing trend in the height of the plant from the landraces to the present cultivars (fig. 3B). The seed size show an increasing trend in the seed size from cultivars (before 1890) to the present crops (fig. 3C). The chlorophyll content has increased from cultivars before 1890 to the modern cultivars (1971-present)(fig. 3A).

![Comparison of growth parameters among the four different groups of barley cultivars](image)

**Fig 3.** Comparison of (A) chlorophyll content (SPAD units); (B) plant height and (C) seed size, among the four different groups of barley cultivars. * p value <0.05 versus cultivars(before 1890); + p value <0.05 versus cultivars(1890-1940); - p value <0.05 versus cultivars(1941-1970).

### 4.4 Comparison of growth parameters among the Nordic countries

Seed size, chlorophyll content and height were also compared among the Scandinavian countries Sweden, Denmark, Norway and Finland. The accessions from Denmark were found to be associated with higher seed size and low chlorophyll levels (shown in fig 4C and 4A). In
contrast, the accessions from Sweden have higher chlorophyll levels compared to other countries. The accessions from Finland were found to be associated with an increase in plant height when compared to other countries (fig. 4B).  

![Comparison of growth parameters with row types](image)

**Fig 4.** Comparison of (A) Chlorophyll content; (B) Plant height and (C) Seed size, among the four Nordic Countries. * p value <0.05 versus Sweden; + p value <0.05 versus Denmark; - p value < 0.05 versus Norway.

### 4.5 Comparison of growth parameters with row types

Among the 82 barley accessions, there are 50 two row varieties and 37 six row varieties. These barley row varieties showed a significant difference in seed size, height of the plant and chlorophyll content. The seed size and chlorophyll rate has decreased significantly with six row varieties (shown in fig 5C and 5A). On contrary, there is a considerable difference with plant height in row varieties and it was found to be higher in six row type varieties (fig 5B). The obtained results indicate that the barley cultivars with six rows have lower chlorophyll rate and larger seed size. However, concerning plant height, the six row varieties were shorter.
Fig 5. Comparison of growth parameters of (A) Chlorophyll content; (B) Plant height and (C) Seed size, between two row and six row barley varieties. * p value <0.05 versus Two row; + p value <0.05 Six row.

4.6 Determination of mineral concentration in grain

Seeds from the 82 barley varieties were harvested and used to measure the seed size, as well as the contents of N, Fe and Zn. Interestingly, there were no significant variation in the N, Fe, or Zn content among the barley accessions when grouped after time period of origin (Fig. 6) or geographical origin of the cultivars (Fig. 8). When two row and six row cultivars were compared, we found that N and Zn were significantly higher in two row varieties (fig. 7A & 7C), whereas the Fe content was found to be higher in six row cultivars (fig. 7B). Interestingly, the single
Swedish two row barley accession from 1890-1940 had highest content of N, Fe and Zn of all 82 accessions.

Fig 6. Error bars representing the variation of concentration of (A) Nitrogen; (B) Zinc and (C) Iron, in the four different groups of 82 barley lines
Fig 7. Error bar graphs showing the (A) Nitrogen; (B) Iron and (C) Zinc, concentration variation between two row and six row barley lines.
Fig 8. Graphs representing the variation of (A) Nitrogen; (B) Zinc and (C) Iron concentration, among the Scandinavian countries

4.7 Association between grain mineral concentration and seed size

Seeds from the 82 barley varieties were harvested and used to measure the seed size, as well as the contents of N, Fe and Zn. The correlation between the concentration of nitrogen and the seed size for the eighty two different barley accessions was weak. However, the grain nitrogen concentration decreased with increase in seed size (Fig. 9A). The iron and zinc concentration did not show any significant correlation to seed size among the four different barley lines. However, the iron and zinc concentration levels did not change in relation to change in seed size (shown in fig. 9B)
Fig 9. Relation between the grain Nitrogen concentration and seed size (A), Iron and Zinc concentration and seed size (B), among the different barley lines

4.8 Association between grain mineral concentration and senescence

The amount of chlorophyll present in plant was measured 20 days after first spike appeared. The mean values of the chlorophyll content was compared with grain nutrient concentration. To investigate if there were any correlation between chlorophyll content and the levels of Nitrogen, and Zinc in the barley e accessions we plotted the obtained data for all 80 accessions in scattered plots (Fig. 10). However, as shown in Fig. 10 there are no evidence for any correlation between the chlorophyll content and levels of Nitrogen, Iron and Zinc in the harvested seeds.

Fig 10. Comparison of chlorophyll and (A) Nitrogen concentration (B) Iron and Zinc concentration, among the eighty different barley lines
5. Discussion

5.1 Mineral concentration in barley grain

The present study helps to investigate the change in grain protein content and mineral concentration in barley from old to modern crops as a result of plant breeding which is aimed for increased grain production. The concentration of grain nitrogen found in this study are comparatively lower in the modern cultivars (1971-present) than in other three groups of cultivars (landrace, cultivars (1890-1940) and cultivars (1941-1970)) of barley but not statistically significant. In contrast, the mineral concentration is higher in modern cultivars(1971-present) compared to the other above mentioned groups but not significant. In wheat grains, there was a significant decrease in the mineral density of iron and zinc in the cultivars of late 1960s (Fan et al., 2008). In contrast, the current study in barley showed increased mineral density but not statistically significant. The concentration of trace elements such as iron, zinc, copper and magnesium was significantly high since 1940, but then has a downward trend in the wheat grains as shown in Fan et al., 2008. The locus Gpc-B1 (Grain protein content-B1) has multiple pleiotrophic effects on grain protein content and mineral concentration in wheat (Distelfeld et al., 2007). Interestingly, the results from the present study did not show significant difference in barley, like for wheat that has been shown in previous studies. Hence, these findings are not consistent with the results of the wheat lines (Zhao et al., 2009; Fan et al., 2008). More than hundred NAC genes present in Arabidopsis are involved in many developmental process, but only a little portion of the NAC proteins are reported till date (Olsen et al., 2005). Hence there might be a possibility of the genes, other than NAM-B1 among the NAC genes could be involved in regulating GPC variation in barley (Jamar et al., 2010). Therefore, there might be a way for Gpc-B1 gene influence in the variation in the Grain nutrient content of barley.

5.2 Relationship between grain mineral concentration and leaf senescence

The chlorophyll rate was significantly higher in cultivars (1971-present), suggesting the delay of maturity in modern barley cultivars. The concentration of nitrogen, iron and zinc does not correlate with chlorophyll content. But the results of chlorophyll measurement are appropriate to the objective of the study which indicates that many other genes could be involved in regulating the senescence in barley. In barley, a different NAC gene which is similar to wheat NAM-B1 might be located at barley locus (Jukanti et al., 2007). There are several other additional genes has a indirect effect in regulating the leaf senescence phenotype of high GPC barley and N remobilization (Jukanti et al., 2007). Jukanti and Fischer (2008) showed that major QTL on chromosome 6 influences the N remobilization from leaves to developing cereal kernels and also indicated several other genes could be essential for this process. The studies conducted by Uauy et al., 2006 suggests that the mutated NAM-B1 gene in wheat has increased the maturity and helps in translocating the nutrients from leaves to developing grains. The loss of NAM-B1 gene resulted in lower nutrients in wheat (Distelfeld et al., 2007). In barley, a broad understanding of NAC gene regulating the GPC variation must be required to investigate for the other genes. Investigation of the other NAC genes is considered to be more valuable for the further studies (Jamar et al., 2010). Among the row varieties analysed, the senescence rate has
decreased in six row types. On the other hand, the two trace elements of iron and zinc concentration showed increased value among the six row barley varieties.

5.3 Effect of NAM-B1 gene on seed size and height

The seed size produced a significant difference from landrace to modern cultivars (1971-present), have a upward trend among the 82 barley accessions. The seed size does not show correlation with the iron and zinc concentration, but the nitrogen concentration showed weak negative correlation. Also, the correlation of seed size was weak with Iron and Zinc concentration in the wheat grains (Zhao et al., 2009). The NAM-1 gene affects both seed size and nutrient content and found to be lost during early domestication in wheat (Uauy et al., 2006). The obtained results from the study indicates that the wild type allele still persists in the 19th century of landrace crops but the seed size was larger and plant height has reduced. Interestingly, it was found that the semi-dwarf varieties has prevented the shortage of world food production (Zhang et al., 2003). Therefore, the sequencing of the semi dwarf allele will provide a pathway to develop molecular markers for the genetic improvement in plant breeding (Zhang et al., 2003)

5.4 Allelic diversity of HvNAM-1 gene

From the observations made in our present study, the results did not show variation among the landrace and cultivars from different time periods. This was strongly supported by Jamar et al., (2010), no polymorphism was observed among the 11 varieties of Hordeum vulgare studied. Hence, these findings could not provide strong evidence to support the objective of the study. The possible explanation is that the genes other than NAM-1 gene also play a vital role in barley. However, the NAC proteins are dimerized and considered as an important protein participating in developmental process (Ernst et al., 2004). Asplund et al., 2010, showed the existence of wild type allele NAM-1 gene in the 19th century of the cultivated wheat. From Distelfeld et al., 2007, the karl haplotype (‘PT’) existed only in very few accessions of barley resulting in low GPC. Whereas, the Lewis haplotype (‘AA’) prevailed highly among the cultivated accession studied. Therefore, the genes that are tightly linked to HvNAM-1 gene might be partially involved in regulating the grain mineral concentration. Investigation of other related genes like Gpc-B1 is worth concerning in the further research. The molecular markers designed for this study has limited to determine the polymorphism among the barley accession. Hence, this requires better molecular genetic markers to find the existence or absence of a gene in the 19th century cultivars of barley. The genotyping of gene bank material would show clearly whether the ancestral NAM-1 allele is present in existing plant material (Asplund et al., 2010). The sequencing of NAM-B1 gene would be useful to compare the sequence of the 80 Scandinavian barley accessions with the wild type allele.

6 Concluding remarks and Future prospects

Our present study results suggests that the nutrient variation in barley might be due to the expression of other NAC genes and this could be contributing a role in regulating the senescence, seed size and height. Therefore, it is worth investigating the other genes, requires new effective molecular genetic markers and further more experiments like sequencing in the future studies.
7 Acknowledgement

I am very thankful to Johan Edqvist and Matti Leino for the kind support to my research work. In addition, I should convey my sincere thanks to NordGen, Alnarp, South Sweden for generously providing the barley seeds to my work.

8 References


**Appendix 1. Allelic diversity of HvNAM-1 gene (uhb6 and uhb7 markers) amongst the various types of barley accessions**

<table>
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<th>Accession number</th>
<th>Origin country</th>
<th>Subtype</th>
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<th>uhb7</th>
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