Department of Physics, Chemistry and Biology

Bachelor's Thesis

The origin of naked barley (*Hordeum vulgare* L. ssp. *vulgare*) studied by the *nud* gene

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The exact origin of the peculiar naked barley is somewhat illusive. There is a debate whether it has a single, monophyletic origin or a multiple, paraphyletic origin. It is from previous Asian studies on naked barley known that a mutation or a deletion of the nud gene expresses the naked seed phenotype. Not much investigation has been done outside of Asia, least of all in the Nordic countries, on what gives naked barley its character. Therefore this study was set up to examine if the Nordic variant of naked barley shares the same nud allele as the Asian and thus has a close connection with it, or if they have independent mutations. I could confirm that the known alleles of the nud gene do determine the seed character of barley. Most of the results of the PCR genotyping confirmed the phenotype of the tested accessions, both naked and hulled barleys. However, one visually phenotyped naked barley cultivar (NGB4580) still amplified with the known primers that would match the Asian hulled allele, meaning that the Nordic accession NGB4580 of naked barley did not carry the known nud deletion. This suggests that naked barley has arisen independently in Asia and in the Nordic countries.
1 Abstract

The exact origin of the peculiar naked barley is somewhat illusive. There is a debate whether it has a single, monophyletic origin or a multiple, paraphyletic origin. It is from previous Asian studies on naked barley known that a mutation or a deletion of the nud gene expresses the naked seed phenotype. Not much investigation has been done outside of Asia, least of all in the Nordic countries, on what gives naked barley its character. Therefore this study was set up to examine if the Nordic variant of naked barley shares the same nud allele as the Asian and thus has a close connection with it, or if they have independent mutations. I could confirm that the known alleles of the nud gene do determine the seed character of barley. Most of the results of the PCR genotyping confirmed the phenotype of the tested accessions, both naked and hulled barleys. However, one visually phenotyped naked barley cultivar (NGB4580) still amplified with the known primers that would match the Asian hulled allele, meaning that the Nordic accession NGB4580 of naked barley did not carry the known nud deletion. This suggests that naked barley has arisen independently in Asia and in the Nordic countries.

2 Introduction

After the most important cereal crops in the world - wheat, rice and maize - barley (Hordeum vulgare L.) comes as the third runner-up (Manjunatha et al. 2011; Taketa et al. 2008). The globally distributed genus Hordeum L. is of the tribe Triticeae which contain 45 species and subspecies (Manjunatha et al. 2011). Of the domesticated barley cultivars the most common type at maturity has caryopses with the outer hull smooth adherent to the pericarp epidermis (Manjunatha et al. 2011; Taketa et al. 2008; 2004). This is known as covered or hulled barley (Figure 1). There is also a so called free-threshing variant named naked or hulless barley (Figure 1), that seems to be restricted to barley only within the Poaceae family (Taketa et al. 2008; 2004).
The seed morphology, or phenotype, of covered barley is believed to be controlled solely by a single locus $Nud$, for nudum, that is dominant over $nud$ causing the naked phenotype (Taketa et al. 2008). The $nud$ locus is located on chromosome 7H on the long arm (Taketa et al. 2008; Fedak et al. 1972; Scholz 1955). The $nud$ locus resides within an ethylene response factor (ERF) family transcription factor gene. The ERF gene is further located within a 16.680 bp long sequence, that has been deleted in some barleys. This is known as the 17 kb deletion when referring to naked barley which expresses the naked caryopsis. The $nud$ gene, consisting of two exons and one intron with the ERF has a role in a special lipid biosynthesis pathway. A lipid layer has been detected on the pericarp or more specifically to the testa, a layer inside the pericarp epidermis in covered barley (Kakeda et al. 2011), and can be detected 10 days after the anthesis period. No expression of ERF have been observed in hulls or leaves (Taketa et al. 2008). The layer seems to cause the adhesion of the hull to the caryopsis. This lipid layer is missing on the naked barley due to the deletion of the ERF gene and the $nud$ locus (Kakeda et al. 2011; Taketa et al. 2008).

Information from the *Arabidopsis thaliana* $WIN1/SHN1$ gene, which is a $nud$ homolog, can help explain further the phenotype change from covered caryopses to naked caused by the nud mutations in barley (Taketa et al. 2008). The $WIN1/SHN1$ gene in normal plants is expressed in particular in areas of cell separation which suggests that this gene plays important roles in lipid biosynthesis pathways. The $WIN1/SHN1$ gene belongs to one of the 10 subfamilies from the ERF family (Taketa et al. 2008).
Additional support for the idea of hull-caryopsis adhesion comes from the rice gene Os06ERF, which is an ortholog to nud, which was particularly expressed in the developing caryopsis (Kakeda et al. 2011).

For agronomic purposes, covered barley has mainly animal feed values and used for brewing purposes, while the naked barley variant is an important source of human food. It is today consumed worldwide but is most frequently eaten in the Far East Asia, especially in the high ranges of the Himalayas, in Nepal and Tibet where it is a staple food (Manjunatha et al. 2011; Kikuchi et al. 2003) and it accounts for more than 95 % of the domesticated barley there (Taketa et al. 2004). Naked barley is a staple food also in India, Bhutan, China, Japan and Korea and to some extent in Ethiopia. Easy processing with no extensive peeling to remove the hull from the edible part and without pleiotropic deleterious effects on other agronomic characters can be one of the main reasons for selection during domestication of naked barley crop (Taketa et al. 2008; 2006). The abundance of dietary fibers mainly β-glucans (which is an inhibitor of cholesterol synthesis), and key vitamins has made naked barley receive more attention recently as feed and healthy human food especially in North America (Barabaschi et al. 2012; Manjunatha et al. 2011; Tonooka et al. 2009; Taketa et al. 2004; Kikuchi et al. 2003), despite the fact that naked barley is less yielding than the hulled type (Barabaschi et al. 2012). Hullied caryopses, on the other hand, with adherent hulls are also advantageous as they protect the seeds from pathogens or physical damage (Kakeda et al. 2011).

The earliest domestication event in barley is recorded up to 10,500 years before present (yBP) in the Fertile Crescent (Turkey, Syria and the Jordan Valley) and is likely one of the founder crops of Eurasian agriculture (Saisho & Purugganan 2007). Later the six-rowed spikes and the naked barley appeared at about 8500 yBP (Zohary et al. 2012; Manjunatha et al. 2011). The exact origin of Nordic naked barley is uncertain. Some speculation regarding the word “himalayense” or “himmelskorn” say it should been appeared first in Finland from the word himalainen (“jingling”), as a descriptive word from when the spikes were detached from the stems (Ahokas 2006). Naked domesticated barley from different regions of the world, mostly Asian, have shown to have homogeneous DNA sequences at the sKT7 locus (a dominant SCAR marker linked to the nud allele), and this supports the idea of a monophyletic origin of naked barley (Taketa et al. 2004). However, no Nordic accessions were included in this study. No recombination between
sKT7 and the nud gene has been detected so far (Kikuchi et al. 2003). The physical distance between the closest marker sKT9 and the nud gene is estimated at 204 kb (Taketa et al. 2006). Since the easily observed naked trait in barley could be a target for selection, the current distribution of naked barley is unlikely to reflect its genetic geographical origin correctly (Taketa et al. 2008).

My aim was to shed some more light on the origin of naked barley with this study, by including Nordic accessions. To detect if the nud gene had been deleted or if it is intact was the first objective of this work, followed by further examination of the naked barley nud allele. With this in mind a broader picture of the relationship of Nordic cultivars can be shown and clearer hypotheses about the true origin can be stated, such as whether naked barley could have a possible polyphyletic origin.

3 Materials & methods

A total of 18 landrace accessions of domesticated barley (Hordeum vulgare L. ssp. vulgare) were studied (Table 1). Ten out of these 18 were obtained directly from the Nordic Genetic Resource Center (NordGen). Five of the accessions were from outside the Nordic countries, four accessions from Afghanistan and one from Nepal. Both caryopses types (C T) were included in the material (Table 1). The barley seeds were sowed and cultivated in a greenhouse at Linköping university. Approximately 100 mg plant tissue was harvested from each barley cultivar and later used for DNA extractions.

For the extraction a protocol and kit from Qiagen was used (DNeasy® Plant Mini Kit, Handbook 10/2012, Plant Tissue Mini Protocol), with an exception of 75 µl instead of 100 µl Buffer AE at the final elution. This change increased the final DNA concentration in the eluate but reduces DNA yield slightly. Plant tissue was ground with a TissueLyser II (Qiagen) at freq. 1/s; 25 and time 30 s. DNA concentration was quantified with a nanodrop instrument.

All accessions were genotyped to determine the presence or absence of the known 17-kb deletion through two different PCR programs. Accessions that were shown to carry the 17 kb deletion marker (HNB32C2-2) were further amplified with F1+kR2 (HNB32C2-4) and F1+kR1 (HNB32C2-3) which also amplify the nud locus (Table 2).
Table 1. Accessions of barley (Hordeum vulgare L. ssp. vulgare) from NordGen. 
http://www.nordgen.org/index.php/skand/content/view/full/344

<table>
<thead>
<tr>
<th>Accession</th>
<th>Name</th>
<th>Origin</th>
<th>C T – PCR</th>
<th>C T – Observed</th>
<th>C T – according to NordGen</th>
<th>DNA C. ng/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGB277</td>
<td>LÄHDE</td>
<td>Finland</td>
<td>Hulled</td>
<td>Hulled</td>
<td>Hulled</td>
<td>52.5</td>
</tr>
<tr>
<td>NGB469</td>
<td>BJØRNEBY</td>
<td>Norway</td>
<td>Hulled</td>
<td>Hulled</td>
<td>Hulled</td>
<td>-</td>
</tr>
<tr>
<td>NGB4579</td>
<td>GUYMALAYE NØGEN 6-RADET</td>
<td>-</td>
<td>Hulless</td>
<td>Hulless</td>
<td>Hulless</td>
<td>26.3</td>
</tr>
<tr>
<td>NGB4580</td>
<td>-</td>
<td></td>
<td>Hulled</td>
<td>Hulless a</td>
<td>Hulless</td>
<td>18.3</td>
</tr>
<tr>
<td>NGB4613</td>
<td>GAMMEL DANSK</td>
<td>Denmark</td>
<td>Hulled</td>
<td>Hulled</td>
<td>Hulled</td>
<td>64.8</td>
</tr>
<tr>
<td>NGB5091</td>
<td>DEH KUNDI K.249</td>
<td>Afghanistan</td>
<td>Hulless</td>
<td>Mixed b</td>
<td>Hulless</td>
<td>45.2</td>
</tr>
<tr>
<td>NGB5094</td>
<td>ZEBAK K.114</td>
<td>Afghanistan</td>
<td>Hulled</td>
<td>Mixed</td>
<td>Hulless</td>
<td>54.6</td>
</tr>
<tr>
<td>NGB6292</td>
<td>PTILI K.133</td>
<td>Afghanistan</td>
<td>Hulled</td>
<td>Hulless</td>
<td>Hulless</td>
<td>71.2</td>
</tr>
<tr>
<td>NGB6295</td>
<td>DEH KUNDI K.245</td>
<td>Afghanistan</td>
<td>Hulled</td>
<td>Mixed</td>
<td>Hulless</td>
<td>67.8</td>
</tr>
<tr>
<td>NGB6927</td>
<td>Lantkorn /jämtland</td>
<td>Sweden</td>
<td>-</td>
<td>Hulled</td>
<td>Hulled</td>
<td>-</td>
</tr>
<tr>
<td>NGB8229</td>
<td>NOGEN 2-RADET</td>
<td>-</td>
<td>Hulled</td>
<td>Mixed</td>
<td>Hulless</td>
<td>35.3</td>
</tr>
<tr>
<td>NGB9305</td>
<td>NÄCKTE VON NEPAL HIMALAYA</td>
<td>Nepal</td>
<td>Hulless</td>
<td>Hulless</td>
<td>Hulless</td>
<td>48.7</td>
</tr>
<tr>
<td>NGB9516</td>
<td>-</td>
<td></td>
<td>Hulled</td>
<td>Hulled</td>
<td>Hulled</td>
<td>27.2</td>
</tr>
<tr>
<td>NGB13504</td>
<td>Lantkorn Gotland</td>
<td>Sweden</td>
<td>Hulled</td>
<td>Hulled</td>
<td>Hulled</td>
<td>31.1</td>
</tr>
<tr>
<td>NGB15229</td>
<td>KUORETON</td>
<td>Finland</td>
<td>Hulless</td>
<td>Hulless</td>
<td>Hulless</td>
<td>-</td>
</tr>
<tr>
<td>NGB15358</td>
<td>AMBLE A-Sogne-Fjord</td>
<td>Norway</td>
<td>Hulled</td>
<td>Hulless</td>
<td>Hulless</td>
<td>28.6</td>
</tr>
<tr>
<td>Clho 2512</td>
<td>Swedish Hulless</td>
<td>Sweden</td>
<td>Hulless</td>
<td>Hulless</td>
<td>43.3</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>Rolfi</td>
<td>Sweden</td>
<td>Hulled</td>
<td>Hulled</td>
<td>44.8</td>
<td></td>
</tr>
</tbody>
</table>

a-The accession NGB4580 showed a different caryopsis type visually than the PCR result suggested.

b-Mixed types, had seeds of both the caryopses types in the seed bags.

Table 2. Primers used in the study (Taketa et al. 2008).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer name</th>
<th>Forward/Reverse primer</th>
<th>Tm</th>
<th>Length</th>
<th>Primer-pair used for amplify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nud/nud (HNB32C2-2)</td>
<td>wF2</td>
<td>GCTTGCAGTACAGAGCTACTAC (forward)</td>
<td>58°C</td>
<td>785</td>
<td>wF2, deletion</td>
</tr>
<tr>
<td>Nud/nud (HNB32C2-2)</td>
<td>kR1</td>
<td>CCTCACCACTAAACCATGCTG (reverse)</td>
<td>55°C</td>
<td>853</td>
<td>wF2, non-deletion</td>
</tr>
<tr>
<td>nud (HNB32C2-3)</td>
<td>F1</td>
<td>ACATTGTTTAGACGCACAAGG (forward)</td>
<td>52°C</td>
<td>kR1</td>
<td></td>
</tr>
<tr>
<td>nud (HNB32C2-4)</td>
<td>kR2</td>
<td>GAGTTGTTGCCACCTGAG (reverse)</td>
<td>56°C</td>
<td>F1</td>
<td></td>
</tr>
</tbody>
</table>
For the PCR the following reagents were used: 15 µl ddH₂O, 2 µl 10xDreamTaq Buffer (containing MgCl₂), 0.4 µl dNTP (10 mM), 0.2 µl forward primer (10 µM), 0.2 µl reverse primer (10 µM), 0.2 µl DreamTaq polymerase (5U/µl) and in addition to the master mix 2 µl template DNA to a final volume of 20 µl (Table 2).

The PCR machines were programmed as followed:
Step 1: 94° C in 2:30 min for one cycle, this initial first step denature the template DNA and activates the polymerase.
Step 2: 94° C in 30 sec (denaturing).
Tm 46° C or 42° C for 30 sec (annealing).
72° C in 1 min/kb (55 sec used) (elongation).
Step 2 was repeated 35 cycles.
Step 3: 72° C in 10 min.
And then a final hold at 4 °C for infinity.

Between the various PCR runs the annealing temperature was optimized for the different primers used. The temperature was optimized to 46 °C for the deletion primer kR1 and 42 °C for the non-deletion primer tR2.

**Gel electrophoresis** (Power Pac Basic, Bio Rad) was run with gels containing 100 ml 0.5x TBE Buffer, 5 µl Cyber Safe® and 1 % agarose at 90-110 V. For each 10 µl of PCR sample a 2 µl loading dye were added. A 100 bp ladder (GeneRuler 100 bp Plus Ladder Thermo scientific) was used as a size reference. The gels were viewed under UV light after they had run for about 45 min, depending on the size of the gel.

Successfully amplified PCR products were further used for **sequencing**. The ExoTAP protocol was used to remove excess dNTP and primers. For each 10 µl PCR product the following reagents were used: 0.01 µl Exonuclease I (20 U/µl), 0.1 µl Thermo sensitive alkaline phosphatase (1 U/µl) and 3.89 µl milliQ-water. After the products had been cleared of excess dNTP and primers, 5 µl PCR product and 5 µl primer (100 µM), were added to the sequencing-plate and sent to Macrogen, the company used for the sequencing [http://www.macrogen.com/eng/](http://www.macrogen.com/eng/). The sequence alignment was performed in the software Geneious, Biomatters Ltd. [http://www.geneious.com/](http://www.geneious.com/).
4 Results

Three accessions, NGB15229, NGB469 and NGB6927, did not germinate and were consequently not used for analysis in this study. The rest of the accessions did germinate and were further used for PCR and sequencing (Table 1). Each accession was genotyped through two runs of PCR, one to amplify the deletion mutant, naked barley accessions, and one to amplify the non-deletion, hulled phenotype. All accessions were successfully amplified with either one or the other primer-pair (Figure 2). Most accessions amplified with the primer pair expected from their observed phenotype. However the accession NGB4580 did not amplify as expected. The documentation from NordGen described this accession NGB4580 as of the naked barley kind which was also confirmed by visual inspection. In spite of this it did not amplify with the deletion primer-pair but instead with the non-deletion primer-pair. The accessions NGB5091, NGB4579, NGB9305 and S.Hulless were further amplified for additional parts of the nud locus (Figure 3). This result indicates that the expressed accessions are of naked barley variants, which shows a amplified locus (HNB32C2-3/4) on the nud locus.

![Figure 2. Results of the PCR. Top row: result of deletion primer-pair wF2+kR1 with amplification for NGB5091 (well 11), NGB4579 (well 13), NGB9305 (well 15), and S.Hulless (well 16). Bottom row: result of the non-deletion primer-pair wF2+tR2 with amplification for NGB15358 (well 2), NGB4613 (well 3), NGB277 (well 4), NGB13504 (well 5), NGB6292 (well 6), NGB6295 (well 7), NGB8229 (well 8), NGB9516 (well 9), NGB5094 (well 12), NGB4580 (well 14), Rolfi (well 17) and in the final well 18 a none template control (NTC). Well 1 and 10 contain a 100 bp ladder respectively.](image-url)
Figure 3. Results of amplification for the locus HNB32C2-3 with primer-pair F1+kR1 (wells 2-9) and the locus HNB32C2-4 with primer pair F1+kR2 (wells 11-17) respectively. Successful amplification was obtained for the naked barley accessions NGB5091 (well 2 and 11), NGB4579 (well 4 and 13), NGB9305 (well 6 and 15), and Sw. Hulless (well 7 and 16).

All the accessions that had been successfully genotyped through the PCR with the deletion fragment 785 bp, the non-deletion fragment 853 bp, the marker HNB32C2-3 or by the marker HNB32C2-4 were sent to Macrogen for sequencing. The sequences were manually inspected and cleaned and later aligned using the software Geneious. The accessions NGB5091, NGB4579, NGB9305 and S.Hulless found after a Linnaeus Blast a match of 386 sequences and a top hit by the ID number AP009567. This match was shown to belong to domesticated barley (Hordeum vulgare L. ssp. vulgare) and matched the nud gene, which revealed a desirable result (Figure 4 and Figure 5).

Figure 4. BLAST result for accession NGB5091 sequenced with forward primer F1 and reverse primer kR2 respectively.
Figure 5. Close-up from Figure 4 showing that the top match to be accession number AP009567, belonging to barley (H. vulgare ssp. vulgare), the nud gene when run through BLAST, [http://blast.ncbi.nlm.nih.gov/](http://blast.ncbi.nlm.nih.gov/).

5 Discussion

To date, no naked wild-type barley has been found in nature or in archeological remains (Zohary et al. 2012). That today’s barley was domesticated from its wild ancestor (H. vulgare ssp. spontaneum), which has hulled caryopses, brittle rachises and two-rowed spikes is generally accepted. Since the domestication of barley (H. vulgare ssp. vulgare) a change to naked caryopses, non-brittleness and six-rowed spikes has emerged (Taketa et al. 2004). Wild barley with six-rowed spikes (H. vulgare ssp. agricriothon) is currently considered a hybrid between ssp. spontaneum and six-rowed domesticated barley and is often normally treated as “wild”.

Analysis of an AFLP from the locus sKT7, closely linked to the nud locus conducted by Taketa et al. (2004), revealed four different alleles I, II, III and IV. All four alleles were found in wild cultivars from the Middle East, which indicated that this region is the center of genetic diversity in wild barley since this region is the only one that still possesses all four alleles (Taketa et al. 2004). The allele IV is rarely found among the wild barley but was found in 100 different landraces of naked barley across Asia and Taketa et al. (2004) concluded here a monophyletic origin of naked barley likely to southwestern Iran where the allele IV was found in a wild barley (accession OUH625). This theory is supported by archeological remains of naked domesticated barley found carbonized (dated 6000 B.C.) in the remains of Ali Kosh (southwestern Iran), but also in Tell Aswad and Tell Abu Hureyra (both Syria) within the Fertile Crescent (Zohary et al. [http://blast.ncbi.nlm.nih.gov/](http://blast.ncbi.nlm.nih.gov/)).
This molecular analysis of the sKT7 locus tightly linked to the nud gene in naked barley suggests that today’s naked barley can be traced back to a single mutation, causing hulless caryopses. It is, however, difficult to conclude whether naked barley derived from wild barley directly or from hulled domesticated barley. The domestication of naked barley from wild, hulled barley would have required two independent mutations (hulled to naked and brittle to non-brittle). Since such double mutations are rare it is more likely that naked barley derived from hulled domesticated barley instead of wild barley (Taketa et al. 2004).

To achieve higher resolution of the nud locus more specific markers and larger population sizes are necessary. Crosses between the hulled and naked barley accessions has been performed to try and map further the genetic structure around the nud allele (Taketa et al. 2006). A gene has been found in barley designated as bgl (1-3, 1-4 –β–D-glucanless grain). It affects the β-glucan content in grains and hardness and is related to endosperm cell walls. Linkage has been found between bgl and the nud gene in naked caryopses, it was mapped to the centromeric region of chromosome 7H (Tonooka et al. 2009). This is an important gene for the grain nutrition but its influence on the naked barley character is uncertain.

Previous molecular evidence has suggested that barley was domesticated once, from populations in the eastern part of the Fertile Crescent (Taketa et al. 2004). According to more recent evidence indicates that a second domestication may have occurred in Central Asia at the eastern edge of the Iranian Plateau or in the western foothills of the Zagros Mountains (Morrell & Clegg 2007). When reviewing ten different geographic regions over Eurasia and North Africa barley accessions can be sub-divided into six different clusters, that Saisho & Purugganan (2007) showed different patterns of variation between different landraces. For that study two different genes were selected to serve as molecular markers Bmy1 and bah45n12 and the analysis showed that Europe or North Africa compared to the Near East have the greatest levels of nucleotide diversity in domesticated and wild barley (Saisho & Purugganan 2007). The distributions of distinct difference of data from eastern to western barley support the migration from at least two independent domestications. One in the Fertile Crescent and the other around the edge of the Iranian Plateau (Morrell & Clegg 2007). The European and North African barley mainly originated from the former and a large part of the Asian barley originated from the latter about 8000 years ago (Morrell & Clegg 2007; Saisho & Purugganan 2007). It might be so that the eastward dispersion of barley
could have followed the trade path or the silk road as it has been called, between Southwest Asia and China, which is known to been utilized for at least 2500 years. Also a westward spread of this new naked barley type is indicated by genetic evidence (Zohary et al. 2012).

The two closely linked loci responsible for non-brittle ears differs as well as allele frequencies among landraces from eastern and western Asia, suggesting independent origins (Morrell & Clegg 2007). This is in contrast with the presence of the hulless barley that is assumed to be controlled by a single locus that suggests a single origin, believed to be somewhere east of the Fertile Crescent (Morrell & Clegg 2007; Taketa et al. 2004). Archeological remains of naked barley have, however, also been found in Finland dated to 3200 +/- 170 B.P. (Ahokas 2006). The history of naked barley roots deep within the Nordic countries have lead to a suggested independent Finnish origin in naked barley or that it was introduced to the Nordic countries by the Finns (Lindtorp 1940).

The barley accession NGB4580 were in this study shown not to have the deletion allele indicative of a hulless phenotype, although visual inspection showed it to be of the naked barley type. This indicate it did not carry the known hulless mutation and contradicts previous conclusions that naked barley should have a monophyletic origin. This study shows instead that Nordic naked barley could have an independent origin. This raises the question of whether it is solely the nud gene that controls the caryopses character or are there more genes involved? Or if the primers used in this study were specific enough to locate the known nud gene? Data about the accessions, such as row type and origin, were not always available from the gene bank and this in turn makes it challenging to assign correct data to them all. Visual examination of the accessions showed some bags to contain mixtures of hulled and hulles seeds of barley and some also contained oats seeds, although they were supposed to contain single accessions. This questions the validity of the accession labeling in the first place. Higher number of properly phenotyped accessions would be desirable for future studies to increase the statistical power of the investigation.
Conclusion

The answer to the question of a single or multiple independent domestication sites of barley depends on what trait you are looking at. The caryopsis type, hulless or hulled barley was thought to have a single origin but the result from this study suggests there are at least two different mutations for hulless barley. The naked barley have later spread to be selected for non-brittle ears and kernel row type that suggests at least two domestications. Today other characters can be seen in barley as well such as seed color and spike density (Assefa & Labuschagne 2004). Although it is surprising that some few early much observant farmers selected a single advantageous mutation of the complete deletion of the nud gene and then later spread worldwide, this study shows the existence of other hulless accessions that do not share the hulless mutation of the Asian cultivars. This study shows that naked barley could have several independent origins, but it is impossible to predict exact geographical where this mutation arose with the few cultivars I had at my disposal during this study.
6 Acknowledgment

To my lab-instructor Maria Lundström, to Matti Leino for the providing of the barley accessions and the pictures within this thesis and not least to Jenny Hagenblad for the tutoring and for the providing of some of the background material.

7 References


Lindtorp O. (1940) Finnskogens folk. Suomen muinaismuistoyhdistys. Finland


