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ORIGINAL ARTICLE

The introduction history of *Hordeum vulgare* var. *nudum* (naked barley) into Fennoscandia

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Abstract

Hordeum vulgare var. *nudum* (naked barley) is one of the oldest and most common cereals found from Neolithic Fennoscandia. After the Bronze Age, naked barley largely disappeared and was replaced by *Hordeum vulgare* var. *vulgare* (hulled barley) and other cereals. During the early 19th century, naked barley of Asian origins was reintroduced to Fennoscandia. In this study, we have genetically characterized samples of Fennoscandian landraces of naked barley which were preserved in gene banks and museum collections. The analyses show that the Fennoscandian naked barley can be split into three groups: First, naked two-row barley, with a likely origin in Asia; second, naked six-row barley, with a likely origin in the eastern Himalayas and introduced during the 19th century; third, naked six-row barley genetically related to the original Fennoscandian hulled barley. The results suggest that this last group represents the ancient form of naked barley, which was possibly introduced in the Neolithic. At that time both naked and hulled barleys were grown and enough gene flow probably occurred between these two subspecies to create a Fennoscandian barley that is genetically distinct, irrespective of whether it is hulled or naked. This hypothesis was further supported by genotyping of the *Nud* gene, which is responsible for the naked phenotype. All naked barleys which we studied contained the same mutation allele, *nud1.a*, thus showing that naked Fennoscandian barley arose by crossings between naked and hulled barley and not by new mutations of hulled barley.

Keywords Naked barley · *Hordeum vulgare* · *Nud1* · Landrace · Fennoscandia · Historic DNA

Introduction

The domestication of *Hordeum vulgare* L. (barley) began around 10,500 years BP (Zohary et al. 2012). Genetic analyses of wild barley and barley landraces (locally cultivated material maintained by farmers) suggest at least two domestication events, one in the fertile crescent of western Asia and one more eastern, possibly in the area of the Zagros mountains, east of the Iranian Plateau (Morrell and Clegg 2007; Saisho and Purugganan 2007). An additional centre of domestication has been suggested in Tibet (for example Dai et al. 2012), but was questioned by Zeng et al. (2018). A history of multiple domestication events is also supported by the genes *btr1* and *btr2* with independent causative mutations for a non-shattering rachis phenotype, and with selection events separated in time and space (Pourkheirandish et al. 2015). Analyses by Poets et al. (2015) further demonstrate that several populations of wild barley contributed to the gene pool of cultivated barley during the domestication process, resulting in environmentally adapted subpopulations.

Whereas wild barley is two-rowed and hulled, domesticated forms of barley also include six-row and naked (hulless) types. The mutations causing these traits appear to be very old. Barley of the six-row type, with spikes with three fertile spikelets on each rachis node instead of one, appear in the archaeological record from 8,800 to 8,000 years BP (Helbæk 1959). At least four alleles (different forms)

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are known of the causative gene *vrs1* which result in six-rowed plants (Komatsuda et al. 2007; Saisho et al. 2009), suggesting that the mutation has occurred repeatedly and then plants with this trait were selected. Naked barley, that is barley grains without adhering hulls (outer lemma and inner palea), have been discovered from around 8,000 years BP. Two naturally occurring alleles of the causative gene for nakedness *nud* have been identified. The first, and by far most common one, *nud1.a*, consists of a 17 kb deletion including the complete *Nud* gene (Taketa et al. 2004, 2008). The second one, *nud1.g*, has only been found in a few naked barleys from Tibet and is caused by a non-synonymous SNP (single nucleotide polymorphism) mutation (Yu et al. 2016).

Although naked barley occurs worldwide, the distribution of the hulled and naked forms varies greatly in time and space. At present, very little naked barley is grown in Europe and the Near East, whereas in eastern Asia and especially the Himalayan highlands, it can be very frequent (Takahashi 1955; Lister and Jones 2013). However, archaeological evidence clearly shows that naked barley used to be much more common in prehistoric Europe and the Near East. In a compilation of archaeological barley finds, Lister and Jones (2013) showed that for the Neolithic and Bronze Age around 40% of sites with barley finds had either naked barley or a mix of naked and hulled forms. For the Iron Age, the frequency of sites with naked barley drops markedly. This

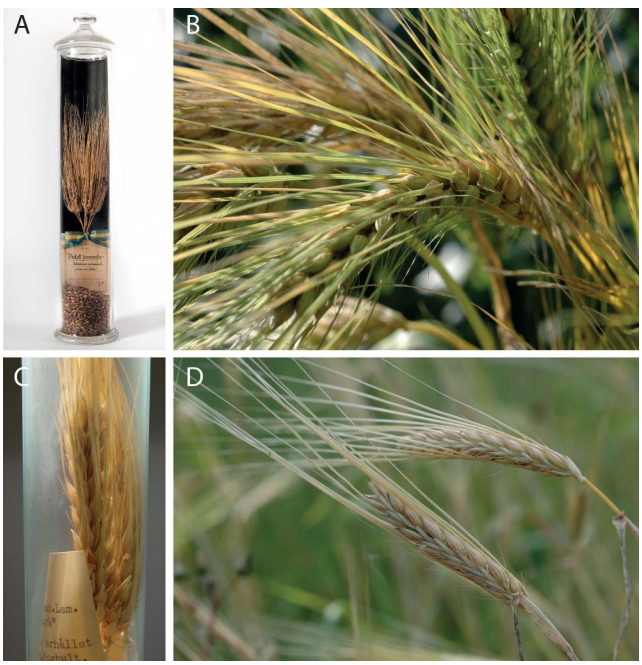

Fig. 1 Examples of materials used in the analyses. **A**, sample of "*Himmels-korn*" (celestial barley) grown in 1894 on the experimental field in Stockhom (accession NM.0405688); **B**, Extant six-row naked barley (NGB 15229); **C**, sample of "Nakenkorn från Sörmark" (naked barley from Sörmark) from 1947 (Å128); **D**, Extant two-row naked barley (CIho 2512). Photos A, Peter Segemark; B, D, Matti W Leino; C, Peter Henriksson

transition from naked to hulled barley is most pronounced in the Nordic countries. In southern Scandinavia naked barley was the main cereal grown during the Neolithic and Bronze Ages, but it was replaced by hulled barley during the transition between the late Bronze Age and pre-Roman Iron Age (Grabowski 2011). Regionally, as in parts of Jutland, Denmark, the cultivation of naked barley lasted well into the Roman Iron Age (Grabowski 2013). In Finland, finds are fewer, but they indicate that naked barley started to decrease during the late Iron Age (Vanhanen 2019).

The reasons for the transition from naked to hulled barley are not clear. The barley hull (husk) is inedible and must be removed if the grain is intended as food for humans. Dehusking of hulled barley requires much work and as yields of naked and hulled barley are in the same range (Barabaschi et al. 2012), it seems peculiar that the Nordic prehistoric societies so clearly changed from naked to hulled barley. Grabowski (2011) suggests various reasons: Hulled barley can resist abiotic and biotic stress better, it responds better to manuring, it is good for animal feed, it is suitable for brewing beer, is more resistant to shattering when harvested with tools such as metal sickles and it stores better, as the husk provides protection against fungal or insect attacks. Whatever the cause, only occasional remains of naked barley have been found in the Nordic countries from after the Iron Age.

Whether the ancient Nordic naked barley completely disappeared is, however, unclear. Historical records from the early modern period mention naked barley sporadically and as a curiosity (Ahokas 2006; Leino 2017). In the 19th century a general belief was that seed taken from areas with a harsh climate would perform particularly well if grown in an area with better conditions (for example Lundequist 1855). Therefore, naked barley seed corn was imported from the highlands of Asia. The material was multiplied and distributed to farmers by the Royal Swedish Academy of Agriculture (Carling 1832; Leino 2017). Seed samples from the academy's experimental fields in the 19th century have been preserved to this day (Fig. 1A). During the 20th century, Fennoscandian naked barley was collected by agronomists and gene banks (Fig. 1B-D; Åberg 1940; Ahokas 2006; Leino 2017). These collections are now curated as non-viable grain samples or as viable germplasm by the Nordic Genetic Resource Center (NordGen).

The identity of the Fennoscandian germplasm of naked barley is, however, unknown. By genetically comparing extant material of naked and hulled barleys from Fennoscandia kept alive in gene banks and from museums and herbaria with that from the mountain regions of Central Asia, we have investigated whether the Fennoscandian germplasm is descended from the 19th century seed imports from Central Asia or is the remnant of an ancient population of

Fennoscandian naked barley. In addition, we have screened the material for presence or absence of the 17 kb deletion of *Nud* to determine whether the nakedness in Fennoscandian barleys is caused by the same deletion allele as in most other naked barleys worldwide. Based on these analyses we propose a history for Fennoscandian naked barley.

Materials and methods

Plant materials

A total of 34 accessions were studied (Table 1). These were chosen to represent both naked and hulled barleys from both the Central Asian highlands of Afghanistan, Nepal and China, and from Fennoscandia (Denmark, Norway, Sweden and Finland). Both two- and six-row accessions were included. Accessions were obtained either from gene banks (the Nordic Genetic Resource Center, NordGen, prefix NGB and the National Small Grains Collection in the United States Department of Agriculture, USDA, prefix Clho) or from historical collections consisting of 19th century seed at the Nordic Museum (prefix NM) (Leino et al. 2009) and the Åberg collection from the 1950s at NordGen (prefix Å). The historical specimens consisted of non-viable harvest grain samples and some specimens also contained ear samples from which the row type (two or six) was determined. When ears were not present, the row type was determined from

Table 1 Accessions included in the study, their origin, row-type, hulledness phenotype, Nud genotype and genetic diversity

Accession	Origin	Source	Row-type	Hulledness	# grains	*Nud* genotyping	*Ppd-H1* genotyping	Diversity (h)
NGB 8868	Afghanistan	Genebank	6	Hulled	5	*Nud*	*Ppd-H1*	0.094
NGB 8872	Afghanistan	Genebank	6	Mix[a]	5	Mix[b]	*Ppd-H1*	0.096
NGB 9605	Afghanistan	Genebank	6	Hulled	5	*Nud*	Mix[c]	0.266
NGB 6292	Afghanistan	Genebank	6	Hulled	4	*Nud*	Mix[d]	0.085
NGB 6295	Afghanistan	Genebank	2	Hulled	4	*Nud*	*Ppd-H1*	0.131
NGB 4671	Afghanistan	Genebank	2	Hulled	5	*Nud*	*Ppd-H1*	0.220
NGB 5091	Afghanistan	Genebank	2	Hulled	4	*Nud*	*Ppd-H1*	0.220
NGB 5094	Afghanistan	Genebank	6	Naked	4	*nud1.a*	*Ppd-H1*	0.060
NGB 9305	Nepal	Genebank	6	Naked	5	*nud1.a*	*ppd-H1*	0.027
NGB 7320	China	Genebank	6	Naked	5	*nud1.a*	*Ppd-H1*	0.063
NM.0405685	Nepal	Museum	6	Naked	5	*nud1.a*	*Ppd-H1*	0.012
NGB 9529	Denmark	Genebank	6	Hulled	6	*Nud*	Not genotyped	0.003
NGB 468	Norway	Genebank	6	Hulled	6	*Nud*	Not genotyped	0.082
NGB 2072	Norway	Genebank	6	Hulled	6	*Nud*	Not genotyped	0
NGB 15103	Sweden	Genebank	6	Hulled	6	*Nud*	Not genotyped	0.107
NGB 27	Finland	Genebank	6	Hulled	6	*Nud*	Not genotyped	0.078
NGB 321	Finland	Genebank	6	Hulled	6	*Nud*	Not genotyped	0.199
NGB 4613	Denmark	Genebank	2	Hulled	5	*Nud*	*ppd-H1*	0.158
NGB 2565	Sweden	Genebank	2	Hulled	5	*Nud*	*ppd-H1*	0.146
NGB 9472	Sweden	Genebank	2	Hulled	5	*Nud*	*ppd-H1*	0.024
NGB 4579	Denmark	Genebank	6	Naked	5	*nud1.a*	*ppd-H1*	0.012
NGB 4580	Denmark	Genebank	6	Naked	5	*nud1.a*	*ppd-H1*	0.012
NGB 15229	Finland	Genebank	6	Naked	4	*nud1.a*	*ppd-H1*	0.012
NM.0406217	Finland	Museum	6	Naked	5	*nud1.a*	*ppd-H1*	0.024
Å128	Sweden	Museum	6	Naked	5	*nud1.a*	*ppd-H1*	0.012
Å147	Sweden	Museum	6	Naked	5	*nud1.a*	*ppd-H1*	0.012
Å353	Sweden	Museum	6	Naked	5	*nud1.a*	*Ppd-H1*	0
Å360	Sweden	Museum	6	Naked	5	*nud1.a*	*Ppd-H1*	0.012
Å363	Sweden	Museum	6	Naked	5	*nud1.a*	*Ppd-H1*	0.012
NM.0406615	Sweden	Museum	6	Naked	5	*nud1.a*	*Ppd-H1*	0.039
NM.0405688	Sweden	Museum	6	Naked	5	*nud1.a*	*Ppd-H1*	0.012
NM.0406621	Finland	Museum	6	Naked	5	*nud1.a*	*Ppd-H1*	0.022
NGB 8229	Denmark	Genebank	2	Naked	4	*nud1.a*	*ppd-H1*	0
Clho2512	Sweden	Genebank	2	Naked	5	*nud1.a*	*ppd-H1*	0

[a] 4 Hulled, 1 naked

[b] 4 *Nud, 1 nud1.a*

[c] 2 *ppd-H1*, 3 *Ppd-H1*

[d] 1 *Ppd-H1*,3 *ppd-H1*

the ratio of straight to twisted grains according to Jacomet (2006). Extant viable accessions were grown and phenotyped for row type and hull characteristics.

DNA extraction

DNA was extracted from four to six individuals of each accession. Extant accessions from gene banks were germinated and DNA was extracted from the resulting leaves using the Qiagen DNeasy Plant Mini Prep Kit according to the manufacturer's instructions. For the historical samples, DNA was extracted from the grains in a sterile hood that had never been used for work with modern material. Individual grains were crushed with sterile pliers and then the DNA was extracted using the MP Biomedicals FastPrep kit. Three rounds of homogenization were used and an extra hour of incubation was included after homogenization to increase the yield. Apart from these modifications, extractions were carried out according to the manufacturer's instructions.

Nud genotyping

All individuals were genotyped for the 17 kb deletion in the *Nud* locus (*nud1.a*) described by Taketa et al. (2008). For extant specimens the *Nud* locus was genotyped with a PCR (polymerase chain reaction) assay according to Taketa et al. (2008), using two sets of primer combinations positioned either outside or inside the deletion. For the historical specimens where the degraded state of the DNA prevented amplification of the original PCR product, a novel forward genotyping primer, nud_short_F3, was used: 5'-ctacaaagccgtgggaatcg-3'. When used with the Taketa et al. (2008) tR2 (5'-gcggtcctttctttccagt-3') primer on DNA not carrying the deletion (hulled phenotype) the resulting DNA fragment was 246 bp and when used with kR1 (5'-cctcaccacttaaccatgtctg-3') on DNA carrying the 17 kb *Nud* deletion (naked phenotype) the resulting fragment was 168 bp. For each individual sample two separate PCRs were run with the two reverse primers tR2 and kR1 respectively, ensuring that each genotype was confirmed by a positive and a negative PCR result.

PCRs were run in a solution made up of 11.4 µl water, 2 µl Dreamtaq buffer, 0.4 µl 10 µM dNTPs (Thermo Scientific), 2 µl 1 µM of each primer and 0.2 µl DreamTaq polymerase (Thermo Scientific) with 2 µl template DNA added to each reaction. The PCR conditions were an initial denaturation at 95 °C for 2 min 30 s, 24 cycles of denaturation at 94 °C for 15 s, annealing at 56 °C for 40 s and extension at 72 °C for 40 s, followed by a final extension at 72 °C for 10 min.

SNP genotyping

The set of individuals was also genotyped for 85 SNP (single nucleotide polymorphism) markers (ESM Table S1). These were chosen from the genome-wide set of barley markers in the BOPA 1 array (Kota et al. 2008). For a subset of accessions, the causative SNP of the photoperiod response gene *Ppd-H1* (SNP48, Jones et al. 2008) was included. Genotyping was carried out by LGC Genomics (Hoddeston, UK) using the KASP method (He et al. 2014).

Data analysis

The software Structure v. 2.2 (Pritchard et al. 2000) was used to investigate the dataset for population structure. As barley is self-fertilizing and largely homozygous, Structure was run in a haploid setting as suggested by Nordborg et al. (2005). It was run using the admixture model, with the length of the burn-in set to 20,000 and followed by 50,000 iterations to estimate parameters. Ten runs were done for each level of K ranging from 2 to 10. To assess the most appropriate number of clusters and to visualize the results, CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) was run with the Greedy algorithm for $4 < K < 6$ and with the LargeKGreedy algorithm for $K \leq 6$. The number of clusters best describing the data was evaluated from the CLUMPP H' values and ΔK calculated according to Evanno et al. (2005). Results were visualized using Distruct v 1.1 (Rosenberg 2004).

R v. 4.1.2 was used to estimate within-accession diversity, to carry out principal component analysis (PCA) and to test for differences in the amount of within-accession genetic diversity between groups of accessions (R Core Team 2020). The *hs* function of the *adegenet* package was used to calculate H_S as a measure of genetic diversity and the *prcomp* function was used for PCA. In PCA the frequency of each allele at each locus and accession was used. Diversity data was tested for normality with the Shapiro-Wilk test using the *shapiro.test* function.

Results

Genetic diversity

In total, genotyping data were analysed from 170 individuals which had been genotyped for 85 genetic markers (ESM Table S1). The success rate for all markers and also for all individuals was above 70% with an average of 96%. The average success rate in the historical material was 95%. Genetic diversity within each accession, calculated as the expected level of heterozygosity, ranged from 0 in four invariant (genetically identical) accessions to 0.220 in

two hulled accessions from Afghanistan (ESM Table S1). No significant differences in the levels of genetic diversity within accessions could be detected between hulled and naked barley, nor between gene bank and museum material (Mann-Whitney U Test, $p > 0.05$).

Structure analysis

Both ΔK and H' values for the Structure analysis suggested that the distribution of genetic diversity was best explained by a model with two clusters (ESM Table S2). At K = 2 both clusters contained both naked and hulled accessions (Fig. 2A). One cluster (dark blue) contained primarily Fennoscandian six-row barley, both hulled and naked. The other cluster (light blue) contained all forms of studied barley, both Asian and Fennoscandian, six- and two-row, and both hulled and naked forms. Some accessions belonged to both clusters, mainly Asian hulled accessions and two-rowed Fennoscandian hulled barley.

The second highest H' value was obtained for K = 9 clusters (ESM Table S2). At this level of clustering, six- and two-row Fennoscandian hulled accessions formed individual clusters (light blue and red in Fig. 2B, respectively). Six-row Fennoscandian naked accessions fell into either of two clusters (light orange or dark green in Fig. 2B). The dark green cluster was also shared by naked six-row Asian accessions NGB 7320 and NM 214, and to a lesser extent by NGB 5094. Additionally, a few hulled barleys of both six-row and two-row Asian accessions were in the dark green cluster. The two-row Fennoscandian naked accessions formed a separate cluster (pink in Fig. 2B). Besides the dark green clustered naked six-row accessions, the Asian accessions showed a higher level of mixed clustering and mostly formed clusters separate from the Fennoscandian ones. The Asian naked six-row accession NGB 9305, however, mostly belonged to one of the Fennoscandian six-row naked clusters (light orange in Fig. 2B).

PCA

In a PCA of the genotype data, principal components 1, 2 and 3 explained 20.59, 15.48 and 14.60% of the variation respectively. The distribution of accessions along the three first principal components to a large extent reflected the results of the Structure analysis, both at K = 2 and K = 9. The groups observed in the PCA could not be explained by origin (Fig. 3A), hulledness (hulled or naked) (Fig. 3B) or row type (Fig. 3C) alone. Instead, all three aspects need to be considered together. The first cluster (positive end of PC1 in Fig. 3), separated from the other accessions along PC1, PC2 and PC3 was primarily made up of Fennoscandian six-row hulled and naked accessions, corresponding

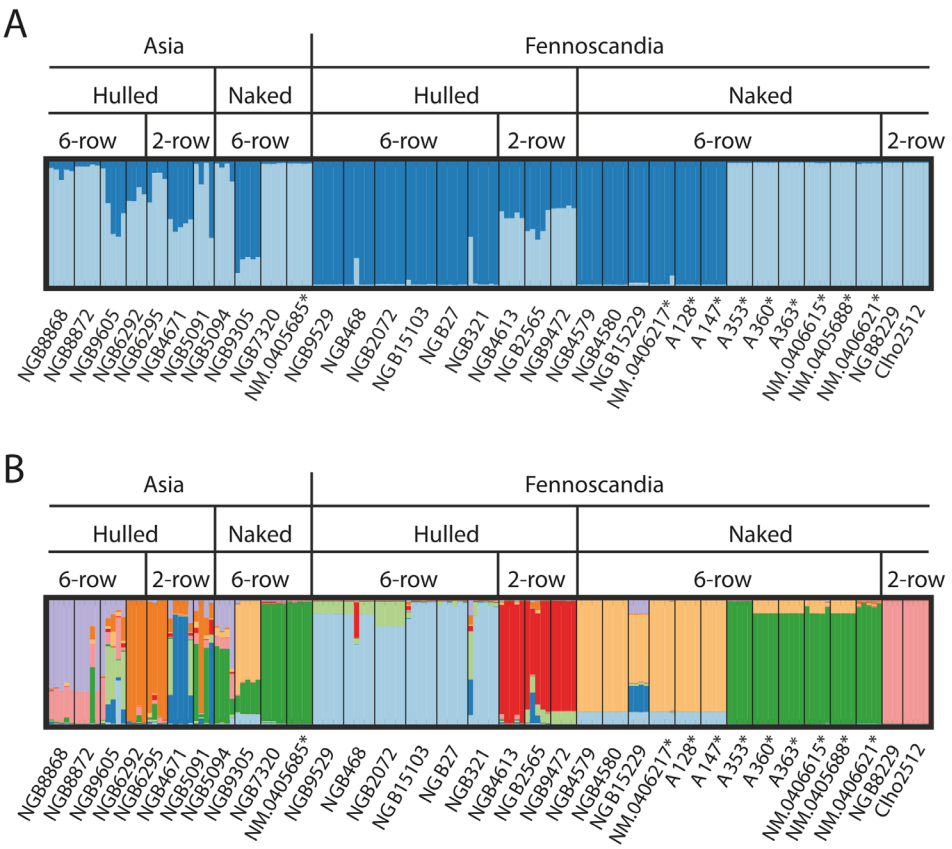

Fig. 2 Results of Structure analyses of Asian and Fennoscandian barleys, **A**, with K = 2; **B**, with K = 9. Each vertical bar represents data from a single individual clustered in accessions separated by thin black lines. Different colours show the proportion of identity of that individual to each cluster (K) explained by the investigated model. The accessions are sorted by origin, hull type and row type. Historical (museum) accessions are marked with *

with the accessions forming the dark blue group at K = 2 of the Structure analysis. Within this cluster, hulled and naked barleys tended to form separate groups (Fig. 3B). The Asian accession NGB 9305 (naked 6-row) also belonged to this cluster but was located away from the hulled and naked subgroups (Fig. 3A). The second cluster (negative end of PC1 in Fig. 3) contained the remaining Asian and several Fennoscandian accessions. Among these, the naked Fennoscandian accessions were both two and six-rowed, while the Fennoscandian hulled accessions were two-row. Within the second cluster, hulled and naked accessions tended to cluster apart along PC3 (Fig. 3B), but no clear grouping could be observed for geographical origin or row type (Fig. 3A, C).

Nud genotyping

Screening for the *nud1.a* allele at the *Nud* locus was done by PCR genotyping according to the Taketa et al. (2008) assay. For the historical samples a new primer was developed, resulting in shorter PCR products which permit amplification of degraded DNA (Fig. 4). All individuals with a naked phenotype were confirmed to carry the 17 kb deletion, the *nud1.a* allele. This deletion was not found in any of the hulled individuals. The accession NGB 8872 phenotyped as a mix of hulled and naked individuals with specimens carrying the corresponding *Nud* and *nud1.a* alleles, respectively.

Discussion

The history of naked barley in Fennoscandia is shrouded in obscurity. Not only are the reasons for the decline of naked barley cultivation after the Bronze Age uncertain, but also the subsequent fate of the crop until the present day is not well known. During the 20th century several accessions of naked barley were collected in Fennoscandia and our genetic analyses of these samples give clues to the history of naked barley in the region.

Two introductions of naked barley to Fennoscandia

Structure and principal component analyses showed that naked Fennoscandian six-row barley belongs to two major groups (Figs. 2A and 3). One of the groups (dark blue in Fig. 2A) showed close similarities to hulled Fennoscandian

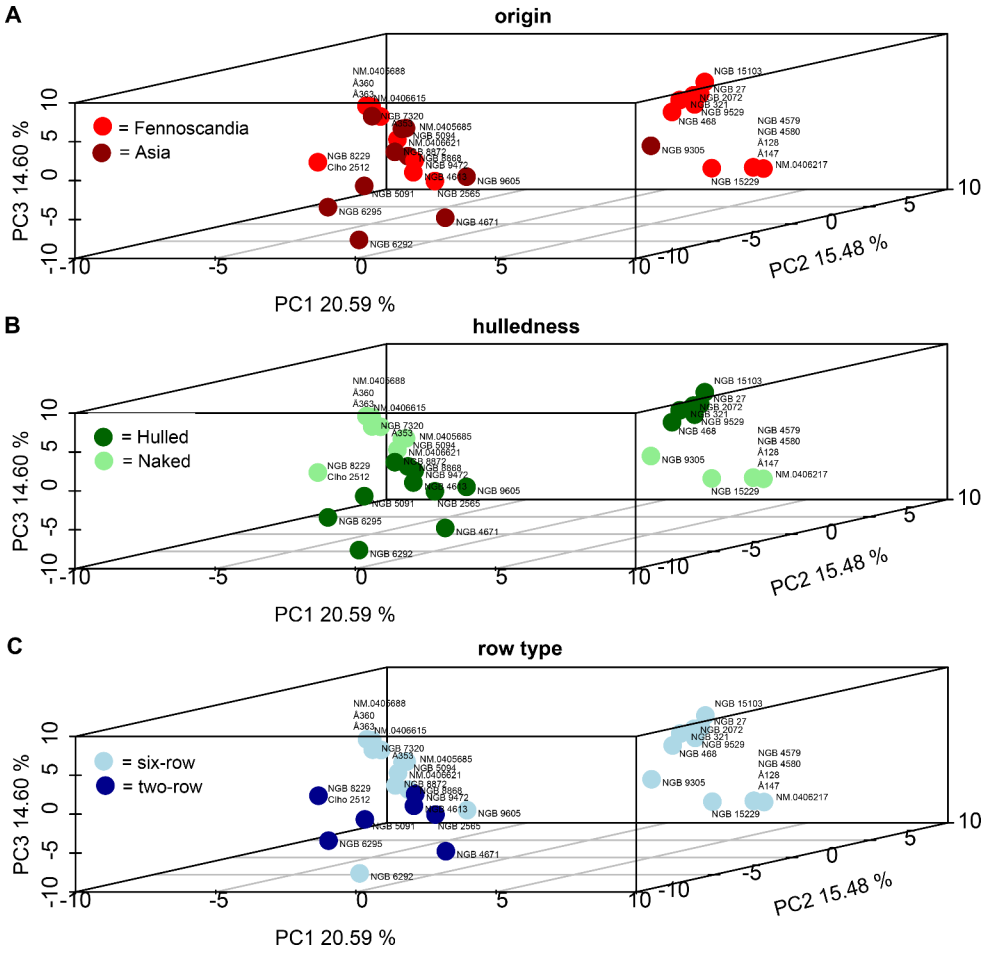

Fig. 3 Three-dimensional principal component analysis (PCA) results of Asian and Fennoscandian barleys. The accessions are coloured after **A**, origin; **B**, hulledness (hulled or naked); **C**, row type

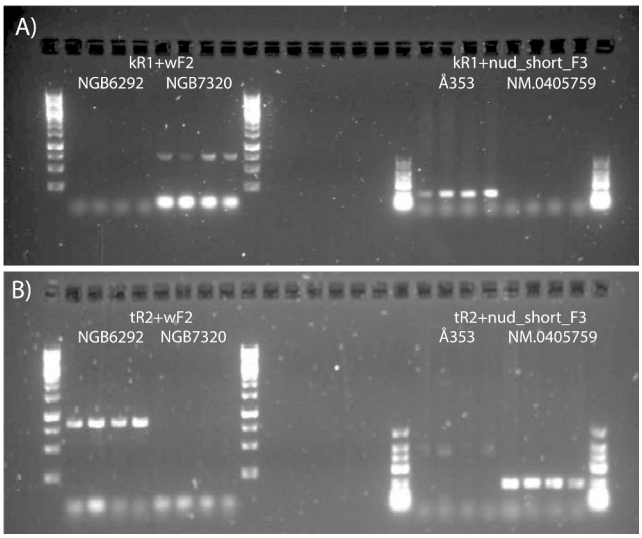

Fig. 4 PCR genotyping results for the *Nud* locus in four individuals each of NGB 6292 (genebank, hulled), NGB 7320 (genebank, naked), Åberg353 (museum, naked) and NM.0405759 (museum, hulled) respectively. **A**, genotyping for *nud.a1* (deletion = naked) allele using the kR1 primer with either wF2 (genebank individuals) or nud_short_F3 (museum individuals); **B**, genotyping for *Nud* (non-deletion = hulled) allele using the tR2 primer with either wF2 (genebank individuals) or nud_short_F3 (museum individuals). The accession NM.0405759 was not used for SNP genotyping and is only included to verify the functionality of the *Nud* genotyping of museum specimens

six-row barley, while the other was more similar to both hulled and naked Asian barleys, both two and six-row. We suggest that these two genetically different groups represent two separate introduction events of naked barley into Fennoscandia.

The original introduction of six-row barley, possibly occurring during the Neolithic, was of both hulled and naked types. At this time both of them were grown extensively in parallel, possibly also mixed, although naked barley was more common (Grabowski 2011; Vanhanen et al. 2019). The fact that the dark blue cluster (Fig. 2A) includes both hulled and naked barley suggests that gene flow must have occurred between the two forms. Over time, a Fennoscandian barley developed that is genetically distinguishable from landraces from other regions, irrespective of whether it is hulled or naked. Further support for the ancient history of this group of naked barleys is given by the presence of the day-length non-responsive allele (*ppd-H1*) of the gene *Ppd-H1* that controls flowering under long day conditions (Table 1). This allele is typical in landraces of barley from northern Europe and has been suggested to originate from the time of the first spread of agriculture in Europe (Lister et al. 2009; Jones et al. 2012). In contrast, all hulled and naked Asian barleys carry the responsive allele of *Ppd-H1*.

Previous investigations of hulled six-row Fennoscandian barley in this region have shown that it is highly distinguishable from all other European barleys, suggesting that it had a long period of adaptation and isolation (Aslan et al. 2015). The current study adds several naked Fennoscandian six-row barleys to this group. These (light orange in Fig. 2B) include both historical and extant accessions from Denmark, Sweden and Finland. Furthermore, principle components analysis shows that they are nearly homogeneous for the studied markers, despite their diverse origins in time and space (Fig. 3). These barleys also have low levels of genetic diversity within each accession (Table 1). A possible explanation for this is that the very limited cultivation of naked barley in Fennoscandia, at least since the 17th century (Ahokas 2006; Leino 2017), led to a reduction in genetic diversity. In addition, conservation of small samples in university collections and in gene banks might have reduced diversity within accessions even further (Hagenblad et al. 2012). Naked barley is very rare, although not completely absent, in archaeological and historical records from the late Iron Age to the 20th century (Viklund 1998; Grabowski 2011; Leino 2017). However, it seems to have been continuously propagated until the time when it was harvested for the historical seed collections and or deposited in the Nordic Genebank.

The second introduction of naked barley to Fennoscandia is reflected in the second group of Scandinavian naked barleys (light blue in Fig. 2A) and likely to be much more recent. This group carries the responsive *Ppd-H1* allele (ESM Table S1) and shows more similarity to Asian barleys than to other Scandinavian landraces as would be expected from a more recent shared ancestry. In general, Asian barleys belong to a different genetic group than northern European (including Fennoscandian) ones (Muñoz-Amatriaín et al. 2014; Pasam et al. 2014). It is thus likely that this second group of naked Fennoscandian accessions originated from the 19th century importing of naked barley from Asia for the experimental field in Stockholm and distribution from there to the rest of Sweden (Carling 1832; Ahokas 2006; Leino 2017).

At higher levels of structuring, this group is split into two sub-groups corresponding to row type (dark green and pink in Fig. 2B). The group of six-row naked barleys (dark green in Fig. 2B) consists mostly of historical material and is very homogeneous. These barleys may possibly all originate from the same batch of seed corn which was imported from Vienna in 1824 as described by Carling (1832). The two-row naked barleys (NGB 8229 and CIho2512), and to some extent also the two-row hulled barleys, are genetically more similar to the Asian barleys than the six-row Fennoscandian barleys (Fig. 3). Comparisons with a more extensive range of naked Asian barleys could shed more light on the origin of the 19th century imports. The results also raise questions about the origin of Fennoscandian two-row hulled barley, a crop type considered to be only a few centuries old in Fennoscandia (Leino 2017).

The different genetic origins of the various samples are also reflected in the confusing nomenclature for naked barley. In Nordic languages, naked barley is frequently named with the prefixes himalais- (meaning dull sound), himmels- (meaning celestial, compare with the former Latin name 'var. *coeleste*') or Himalayan- (meaning originating in the Himalayas, compare with the former Latin name var. *himalayense*) (Ahokas 2006). Although the meanings of the three names are completely different, their spelling and pronunciation are similar, leading to misunderstandings. For example, among our accessions NM217 is named "himmelskorn" and NM1153 is named "Himalaya", although both accessions are genetically identical and of Asian origin. In contrast, NM748 is named "Himalaya" and NGB 9305 is named "Näckte von Nepal" (Naked from Nepal) in spite of being genetically similar to the ancient Fennoscandian type. We thus caution against placing too much trust in accession names for geographical origin, at least in the case of Fennoscandian naked barley.

Fennoscandian naked barleys with the *nud1.a* allele

PCR genotyping confirmed the presence of the *nud1.a* allele in all individuals with the naked phenotype. Previous screenings of barley of world-wide origins have found that the *nud1.a* allele always occurs together with the naked grain phenotype (Taketa et al. 2004, 2008; Lei et al. 2020). Fennoscandian barleys have so far been poorly investigated, but this study confirms the presence of *nud1.a* in barley also from this part of the world. Thus, our results provide further support to Taketa et al. (2008) in suggesting an ancient origin of naked barley from a common ancestor. The only known exception so far is the *nud1.g* allele, which is found at low frequency among naked barleys from Tibet. Our study included several accessions from the central Asian highlands, but mainly from the more western parts. Together, it thus seems that the presence of the *nud1.g* allele is limited to a very restricted area in Tibet.

Conclusions

Our data support an ancient deletion mutation allele, *nud1.a*, that was present in naked barley when it, together with hulled barley, was introduced to Fennoscandia in the Neolithic period. Over time, gene flow between naked and hulled barleys in Fennoscandia has led to homogeneity between the two forms. During the 19th century both six- and two-row naked barleys were introduced from Asia to Fennoscandia. Both the ancient Fennoscandian six-row naked barley and the 19th century introductions have survived to the present day.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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