

Genetic diversity, population structure and linkage disequilibrium in Nordic spring barley (*Hordeum vulgare* L. subsp. *vulgare*)

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Abstract Genetic diversity, population structure and genome-wide linkage disequilibrium (LD) was estimated in Nordic spring barley (*Hordeum vulgare* L. subsp. *vulgare*) by genotyping 180 breeding lines with 48 SSR markers and 7842 high-confidence SNPs using the Illumina Infinium 9K assay. In total 6208 SNPs were polymorphic and selected for further statistical analysis. A Mantel test revealed a strong positive correlation with a Pearson's correlation coefficient (r) of 0.86, between the estimates of genetic distances based on SSR and SNP data. Population structure analysis identified two groups with a clear ancestry and one group with an admixed ancestry. The groups were primarily separated based on row-type and geographical origin. Average LD for the whole population decayed below a critical level of $r^2 = 0.20$ within a range of 0–4 cM. To avoid

confounding effects of the strong population structure, LD decay for the different groups was analysed separately and ranged from 0 to 12 cM. A slower LD decay was found within the two-rowed lines compared to the six-rowed lines and the two-rowed lines originating from the northern part, which could be the result of strong selection for malting quality and yield in the southern part. No large difference in genetic diversity was observed between population sub-groups, but differences at certain chromosomal regions were evident.

Keywords *Hordeum vulgare* L. · Linkage disequilibrium · Microsatellites · Molecular markers · Plant breeding · SNP array

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Introduction

Cultivated barley (*Hordeum vulgare* L. subsp. *vulgare*) is one of the most important crops in the Nordic countries covering a total area of 1.56 million ha in 2014 (<http://faostat3.fao.org>). Nordic barley breeding began more than 100 years ago, starting by selections in the landrace gene pool to the present modern elite cultivars developed by crosses between pure lines of advanced material (Kolodinska Brantestam et al. 2004; Fischbeck 1992). Exotic sources are used in modern breeding, but then primarily as donors of single resistance genes against major diseases

(Melchinger et al. 1994; Weibull et al. 2003). Today's cultivation of genetically uniform cultivars is raising concerns about loss of genetic diversity. A previous study of the genetic diversity in spring barley germplasm in the Nordic and Baltic region reported a significant decrease of genetic diversity in the spring barley from southern parts of the investigated region in the middle of the twentieth century, but not in the spring barley from the northern parts (Kolodinska Brantestam et al. 2007). Likewise, no signs of genetic erosion were observed in a recent study of genetic diversity for barleys from the northern European area over a hundred years of barley breeding (Rajala et al. 2016). This highlights the importance of knowledge regarding the level of genetic diversity in breeding material, since it enables the detection of any changes in diversity that might lead to genetic erosion.

Several types of molecular markers such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), diversity array technology (DArT), and single nucleotide polymorphism (SNP) have been used to study genetic diversity and structure in crops (Kesawat and Das Kumar 2009). SSRs have the advantage to be abundant, highly polymorphic and multi-allelic, and therefore often provide more information compared to biallelic markers such as SNPs. On the other hand, the new iSelect genotyping platform, based on the Illumina Infinium assay, allows the simultaneous testing of 7842 gene-derived SNPs (Comadran et al. 2012). The genetic polymorphism of the SNP and SSR marker systems are generated through different mechanisms, thus they could give different views of the structure of a population.

Analysing the genetic structure within a population is a critical step to the way to understand and reveal the complexity within this population (Pritchard et al. 2000). Factors such as human or environmentally driven selection, genetic drift, mating system and growth habit can have an effect on the population structure (Buckler and Thornsberry 2002; Flint-Garcia et al. 2003). Studies of worldwide (Malysheva-Otto et al. 2006), European (Rostoks et al. 2006), American (Hamblin et al. 2010) and Nordic (Rajala et al. 2016) barley germplasm have shown that cultivated barley has a clear level of population structure with major subpopulations caused by differences in ear type, i.e. two-row and six-row, and seasonal growth habit, i.e. winter and spring (Hamblin et al. 2010; Malysheva-

Otto et al. 2006; Rostoks et al. 2006). In addition to these major subpopulations, it has been shown that American barley accessions further can be divided into minor sub-populations corresponding to the breeding programs, which might be allocated to limited exchange of material between the breeding programs and/or a result of local adaptation (Hamblin et al. 2010).

Another important factor to consider is linkage disequilibrium (LD), which is the non-random association of alleles between two loci and shows the correlation between genetic polymorphisms, e.g. SNPs, and their history of mutations and recombination (Flint-Garcia et al. 2003). LD is important since the rate of its decay in a given species determines the number and density of the molecular markers needed to perform GWAS (Rafalski 2002). In many self-pollinated species such as barley where LD extends over long chromosomal distances (Malysheva-Otto et al. 2006), fewer markers are needed to cover the whole genome, whereas a higher marker density is needed when LD decays very rapidly in species such as maize where it declines to nominal levels within 1.5 kb (Remington et al. 2001).

The aim of the Public Private Partnership (PPP) for pre-breeding in barley, partly funded by the Nordic Council of Ministers (NMR), is to lay a foundation for barley breeding for disease resistance and yield stability to meet current and future challenges in the Nordic region. This collaboration is between five breeding companies and three governmental organizations in the Nordic region. One of the goals with this program is to identify markers linked with traits of interest via genome-wide association studies (GWAS). The main objective with the present study is to determine the population structure and the LD decay in a Nordic barley panel, in order to estimate the relationships among individuals.

Materials and methods

Plant materials

A total of 134 and 46 spring barley *Hordeum vulgare* L. subsp. *vulgare* breeding lines and cultivars, respectively, were included in this study. The selected spring barley breeding lines and cultivars are hereafter referred to as lines. Equal number of lines was selected

by breeders from each of Boreal Plant Breeding (Finland), Graminor Breeding AS (Norway), Agricultural University of Iceland (AUI Iceland), Lantmännen Lantbruk (LSW Sweden), Nordic Seed and Sejet Planteforædling I/S (Denmark). The lines were chosen to represent the available genetic variation in current elite Nordic barley germplasm. Out of the 180 lines, eleven lines were removed from further analyses since they were duplicates, or due to incomplete genotyping. Out of the remaining 169 lines, 124 were two-rowed and 45 six-rowed.

DNA extraction

DNA was extracted from 2-week-old seedlings, using a CTAB (Cetyl Trimethyl Ammonium Bromide) method as described earlier by (Orabi et al. 2014). The DNA was precipitated with isopropanol, washed two times with 75% ethanol, air-dried and finally diluted in TE buffer (pH 8.0).

Microsatellite genotyping

All lines were genotyped using 48 microsatellite markers evenly distributed over all chromosomes. PCR amplifications were performed on a GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA) using a single universal touchdown PCR program as previously described in (Orabi et al. 2014). The forward primers were 5'-labeled with fluorescent dyes to achieve the maximum multiplex capacity of the ABI 3130xl sequencer. Direct and M13-labelling were used for the microsatellite fragments, with 6-carboxyfluorescein (6-FAM, blue) or hexachloro-6-carboxyfluorescein (HEX or VIC, green) and 5'-fluorescein phosphoramidite (NED, yellow) for the direct labelling. For the M-13 labelling, 6-FAM (blue), VIC (green) and NED (yellow) were used. For fragment detection the ABI 3130xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) was used and the fragment analysis and genotyping were performed using the GeneMarker genotyping software program, version 1.85 (Soft genomics, State College, PA, USA).

SNP genotyping

All lines were genotyped with the barley iSelect SNP chip based on the Illumina Infinium 9K assay. The

genotyping of the lines was outsourced to Trait Genetics. The chip consists of 7842 high-confidence SNPs derived from expressed genes (Comadran et al. 2012).

Data analysis

Gene diversity and marker allele frequency

Genetic distances between genotypes, genetic diversity, allele frequency and private alleles (alleles present only in one group) were calculated using an in-house program written in VBA (Visual Basic for Applications) and implemented in Microsoft Excel 2007 (Microsoft, Redmond, WA, USA). The program utilises R language software v.2.14.2 (R Development Core Team 2012), which includes the Modern Applied Statistics with S-plus (MASS) package (Ripley 2002).

The average number of alleles per locus per group represents how polymorphic a given marker was within each group, and this value was calculated for each SNP marker. The average number of alleles per marker is between 1 and 2, where markers with a number of 1 were considered monomorphic.

Modified Roger's distances (MRD) based on (Wright 1978) were calculated for the SSR data based on the following equation:

$$MRD \sqrt{\frac{1}{2m} \sum_{i=1}^m \sum_{k=1}^{a_i} (p_{ij} - q_{ij})^2}$$

where p_{ij} and q_{ij} are the allele frequencies of the j th allele at the i th marker of the two barley lines in consideration; a_i is the number of alleles at the i th marker; and m is the number of SSR loci. The genetic distances (D_{SM}) based on the SNP data were determined by estimating a simple matching coefficient (S_{SM}) (Reif et al. 2005):

$$D_{SM} = 1 - S_{SM}; \quad S_{SM} = \frac{v_{ij} + y_{ij}}{v_{ij} + w_{ij} + x_{ij} + y_{ij}}$$

where v_{ij} refers to the allele in common between two lines, i and j ; w_{ij} is the number of alleles present in i and absent in j ; x_{ij} is the number of alleles present in j and absent in i ; and y_{ij} is the number of alleles absent in both i and j . Correlations between the MRD (SSR) and D_{SM} (SNP) matrices were calculated using Mantel test (Mantel 1967). The polymorphic information content (PIC) of the individual markers was calculated as explained by Botstein et al. (1980):

$$PIC = 1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^n 2p_i^2 p_j^2$$

where p_i is the frequency of the i th allele, and n is the number of alleles per marker.

To show genetic diversity and differentiation along the barley chromosomes Shannon's diversity indices were calculated using GenAIEx v. 6.5.0.1 (Peakall and Smouse 2006, 2012) based on the SNP data.

Population structure analysis

To determine population structure of the barley lines using SNP markers, the software package STRUCTURE v.2.3.4 based on a Bayesian clustering approach, was used (Pritchard et al. 2000). STRUCTURE was run 10 times for each hypothetical number of subpopulations (K) between 1 and 12 with the ploidy level set as 2. The Markov chain Monte Carlo (MCMC) was set to 9999 burn-in phases followed by 9999 iterations. Structure Harvester v.0.6.94 (Earl and von Holdt 2012), was used to estimate the most likely number of groups (K) using the DeltaK method (Evanno et al. 2005). Population structure based on the SSR markers was calculated as described above with the same settings but by using the previously described VBA program in Excel.

Analysis of Molecular Variance (AMOVA), Nei's unbiased genetic distance and Principal Coordinates Analysis (PCoA) were calculated using GenAIEx v. 6.5.0.1.

Linkage disequilibrium analysis

The TASSEL 3.0 software (<http://www.maizegenetics.net>) was used to calculate the LD (allele frequency correlation, r^2) estimates between the SNP marker pairs using the full matrix option. Only intra-chromosomal comparisons were included and markers with minor allele frequency (MAF) below 0.05 were excluded. Thus 4884 out of the total 6280 polymorphic markers were subjected to analysis. To estimate the LD decay, the intra-chromosomal r^2 values were plotted against the genetic distance with a second-degree smoothed loess curve fitted using the program R (R Development Core Team 2012) and a baseline based on the critical value of r^2 was drawn. The critical value of r^2 , as an evidence of linkage, was

calculated based on the method described in Breseghello and Sorrells (2006), by square root transforming the r^2 -values and taking the 95th percentile of unlinked r^2 -values. In the analysis markers located more than 50 cM apart were considered unlinked.

Results

Population structure in Nordic spring barley

The STRUCTURE analysis indicated that the barley panel could be divided into two groups with common ancestry (>0.7) $K1_{SSR}$ ($n = 109$) and $K2_{SSR}$ ($n = 50$) and one group ($n = 10$) with an admixed ancestry (<0.7), based on the SSR data. AMOVA analysis revealed that the $K1_{SSR}$, $K2_{SSR}$ and admixed $_{SSR}$ group were significantly separated ($p < 0.001$) and explained 35% of total molecular variance (Online Resource 1). This is similar to the result obtained from the PCoA analysis, where the first two principal coordinates combined explained 32.1% (21.7 and 10.4%) of the variation (Fig. 1a). The subdivision of the population along the first principal coordinate (PC1) corresponded to the separation of the barley population into $K1_{SSR}$ and $K2_{SSR}$. The $K1_{SSR}$ and $K2_{SSR}$ group corresponded to two-rowed lines and six-rowed lines, respectively, with the exception of six two-rowed lines found in $K2_{SSR}$. In the small admixed $_{SSR}$ group, nine two-rowed lines and one six-rowed line from mainly the northern parts were found.

Also for the SNP data, two groups with a common ancestry (>0.7) ($K1_{SNP}$: $n = 109$; $K2_{SNP}$: $n = 47$) and one group ($n = 13$) with an admixed ancestry (<0.7) were inferred by the STRUCTURE analysis. Three clusters were observed in the PCoA analysis, where the first and second principal components explained 24.6 and 5.9% of the variation, respectively, or 30.5% combined (Fig. 1b). AMOVA analysis revealed that the $K1_{SNP}$, $K2_{SNP}$ and admixed $_{SNP}$ groups were significantly separated (p value < 0.001) and explained 42% of the total molecular variance (Online Resource 1). The two-rowed lines were distributed between $K1_{SNP}$ and the admixed $_{SNP}$ group and the six-rowed lines were found in group $K2_{SNP}$, with the exception of two two-rowed lines that were found in the latter group. The lines with an admixed ancestry in the admixed $_{SNP}$ group were two-rowed and were, just as

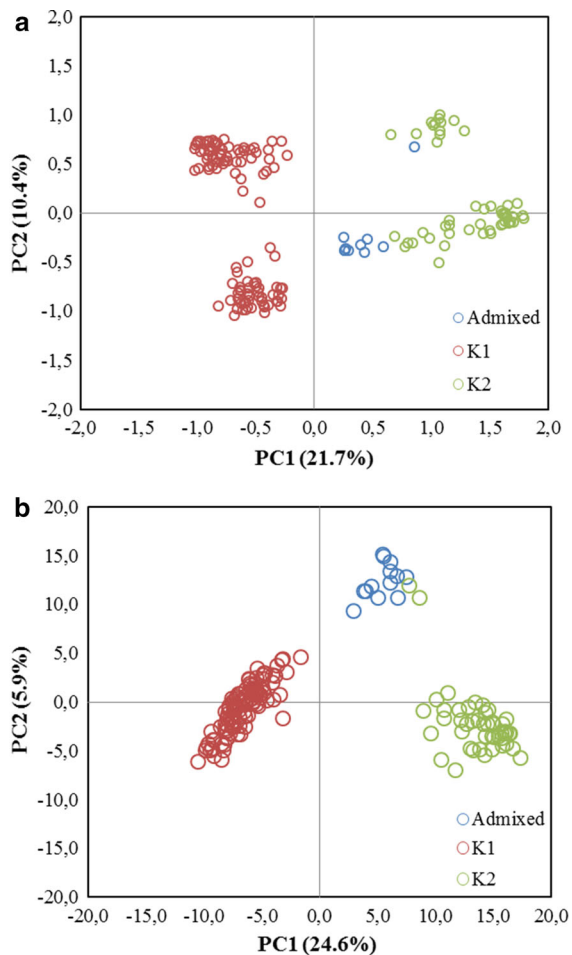


Fig. 1 **a** Associations between structure groups revealed by principal coordinate analysis of the Nordic spring barley collection based on the SSR data. **b** Associations between structure groups revealed by principal coordinate analysis of the Nordic spring barley collection based on the SNP data

the lines in the admixed_{SSR} group, mainly from the northern parts. More detailed information regarding the origin of the lines and the inferred population structure groups can be found in Online Resource 2.

Genetic diversity in Nordic spring barley

Marker system comparison

The barley lines were genotyped using 48 SSR markers. The same lines were also genotyped using the iSelect 9K SNP barley chip resulting in a total of 6208 polymorphic SNP markers. The SSR markers produced 234 scorable loci. The number of alleles per marker ranged from 1 to 15 with an average of 4.9

alleles per SSR marker. The average polymorphic information content (PIC) value was 0.46 for the SSR markers and 0.28 for the SNP markers. The genetic diversity index was higher for the SSR markers (0.514) than for the SNP markers (0.359).

Mantel test showed a strong correlation between the SSR-based Modified Roger's genetic distances and the SNP-based simple matching coefficient, with a Pearson's value of $r^2 = 0.86$ (p value <0.0000).

Genetic diversity based on ear row type

Allelic richness parameters in the form of average number of alleles per locus, number and proportion of private alleles and genetic diversity for each marker type based on ear row type are presented in Table 1. There were no major differences in the average number of alleles per locus found in the two-rowed lines (SSR, 4.0; SNP: 1.9) compared to the six-rowed lines (SSR: 3.5, SNP: 1.8). The genetic diversity was higher in the two-rowed lines (SSR: 0.431; SNP: 0.305) compared to the six-rowed lines (SSR: 0.386; SNP: 0.225). The two-rowed lines had also a higher number of private alleles (SSR: 64; SNP: 1145) compared to the six-rowed lines (SSR: 41; SNP: 447). AMOVA analysis revealed that 35 and 40% of the molecular variance between the lines based on the SSRs and SNPs, respectively, could be explained by the row-types (data not shown).

Genetic diversity based on population structure

Allelic richness parameters in the form of average number of alleles per locus, number and proportion of private alleles and genetic diversity for each marker type based on the inferred population structure groups are presented in Table 2. For the SSRs the highest average number of alleles per locus and gene diversity was found in the admixed_{SSR} group (3.7; 0.404). However, this group also had the lowest proportion of private alleles (0.30), whereas the highest proportion of private alleles was found within the six-rowed lines in group K2_{SSR} (0.86).

No major differences in the average number of alleles per locus were found between the three subgroups, based on the SNP data. The highest proportion of private alleles (10.2) was, no different from the results obtained with the SSRs, found within the six-rowed lines in K2_{SNP} whereas the lowest

Table 1 Allele frequency and genetic diversity in two- and six-rowed barley, based on SSR and SNP data

	Population size (n)	Average number of alleles per locus		Number of private alleles		Proportion of private alleles		Genetic diversity (<i>D</i>)		Standard deviation (<i>D</i>)	
		SSR	SNP	SSR	SNP	SSR	SNP	SSR	SNP	SSR	SNP
Two-row	124	4	1.9	64	1145	0.5	9.2	0.431	0.305	0.063	0.100
Six-row	45	3.5	1.8	41	447	0.9	9.9	0.386	0.225	0.123	0.233
Total	169							0.514	0.359	0.434	0.448

proportion of private alleles (0.54) were found in the admixed_{SNP} group. The highest gene diversity (0.279) was found in K1_{SNP}, whereas the lowest (0.198) was found in the admixed_{SNP} group.

Matrices showing relationships between the structure groups were generated based on Nei's unbiased genetic distance for SSR and SNP data (Table 3). In the SSR matrix, the smallest distance (0.272) was found between the K1_{SSR} and admixed_{SSR} group (both mainly two-rowed lines from the southern and northern parts, respectively). The largest distance (0.566) was found between groups K2_{SSR} (mainly six-rowed lines) and admixed_{SSR} (mainly two-rowed lines from the southern and northern parts, respectively). Similar results were seen in the SNP matrix, where the smallest distance (0.217) was found between groups K1_{SNP} and admixed_{SNP} (mainly two-rowed lines from the southern and northern parts, respectively) and the largest (0.338) between K2_{SNP} (mainly six-rowed lines) and admixed_{SNP} (mainly two-rowed lines from the northern parts).

When comparing genetic diversity along the barley chromosomes, differences between the population structure groups were evident in several genomic regions (Fig. 2). Both group K2_{SNP} and admixed_{SNP} were low in diversity on chromosome 2H (around 83–113 cM), 3H (around 37–64 cM) and 5H (around 108–127 cM), whereas the K1_{SNP} lines were very diverse in these regions. A large difference in genetic diversity were also observed on chromosome 6H (around 55–58 cM), where the K1_{SNP} and K2_{SNP} lines were much more diverse compared to admixed_{SNP} lines. Right before this region on chromosome 6H (around 49 cM), a region with low diversity was seen for the K1_{SNP} lines, but here the diversity was maintained for the two other groups. On chromosome 7H (around 68–88 cM) the lines in group K2_{SNP} were

very diverse in contrast to the low diversity observed for the K1_{SNP} and admixed_{SNP} lines in the same region.

Linkage disequilibrium in Nordic spring barley

LD for the whole population, the population structure groups and the ear row-types were calculated based on the SNP markers. The percentage of unlinked marker pairs ranged between 40 and 42% and no large differences were found between the different groups. The number of intra-chromosomal marker-pairs and the number of unlinked pairs in the total population and in the different groups are presented in Table 4. A total of 1,795,852 intra-chromosomal marker pairs were found in the entire population. The mean r^2 -value of the entire population, the ear row-types and population structure groups were calculated for the whole genome and for the seven chromosomes separately (Table 5). The mean r^2 -value for the whole genome of the entire population was found to be 0.10. The highest and lowest mean r^2 -value for the whole genome was found in the admixed_{SNP} and K1_{SNP} group (r^2 : 0.19; 0.07), respectively. The interval in which the Loess curve intercepts the critical value (background LD) was considered as the LD decay. The background LD and the LD decay of the entire population, the ear row-types and population structure groups were calculated for the whole genome and for the seven chromosomes separately (Table 6, Online Resource 3). Average LD for the whole population decayed below the critical level ($r^2 = 0.20$) within a range of 0–4.0 cM. However the LD decay for the different chromosomes, ear row-types and population structure groups ranged from 0 to 12, with the most extended LD decay found for 5H in the six-rowed lines and in group K1_{SNP} with an average r^2 of 8–12

Table 2 Private alleles and genetic diversity in the structure groups based on SSR and SNP data

Group	SSR data						SNP data					
	Population size	Average number of alleles per locus	No. of private alleles	Proportion of private alleles	Genetic Diversity (<i>D</i>)	Standard deviation (<i>D</i>)	Population size	Average number of alleles per locus	No. of private alleles	Proportion of private alleles	Genetic Diversity (<i>D</i>)	Standard deviation (<i>D</i>)
K1 _{SSR}	109	2.5	42	0.39	0.392	0.275	109	1.9	639	5.86	0.279	0.153
K2 _{SSR}	50	3.5	43	0.86	0.397	0.063	47	1.8	481	10.23	0.235	0.208
Admixed _{SSR}	10	3.7	3	0.30	0.404	0.120	13	1.6	7	0.54	0.198	0.495
Total	169				0.514	0.434	169				0.359	0.448

Table 3 Nei’s unbiased genetic distance between different structure groups, based on the (a) SSR data, (b) SNP data

	K1 _{SSR}	K2 _{SSR}	Admixed _{SSR}
(a)			
K1 _{SSR}	0.000		
K2 _{SSR}	0.292	0.000	
Admixed _{SSR}	0.272	0.566	0.000
	K1 _{SNP}	K2 _{SNP}	Admixed _{SNP}
(b)			
K1 _{SNP}	0.000		
K2 _{SNP}	0.250	0.000	
Admixed _{SNP}	0.217	0.338	0.000

(Table 6). When comparing the whole genome, the six-rowed lines, group K2_{SNP} and the lines from the northern parts in group admixed_{SNP} had a more rapid LD decay with an average r^2 between 0 and 4 compared to the two-rowed lines and group K1_{SNP}, with lines from the southern parts, where the average LD decay were more slow with an average r^2 between 4–8 and 8–12, respectively.

Discussion

Comparison of marker systems

It is of interest to compare if the information regarding population structure and genetic diversity is affected by the marker system of choice, since there are different mutational mechanisms behind SSR (replication slippage) and SNP (point mutation) markers. SSRs have been the most commonly used for studies of genetic diversity, mainly due to their abundance in the genome, reproducibility and high level of polymorphism. However, the increased availability of the SNP markers and the fast and highly automated genotyping technologies, have recently moved the attention to the use of the SNPs in studies of genetic diversity and population structure. This study showed that the average PIC and genetic diversity values were higher for the SSRs compared to the SNPs. However, these values have a maximum value of 0.5 for bi-allelic markers such as SNPs when the markers scores are 50% (0) and 50% (1). Taking this into

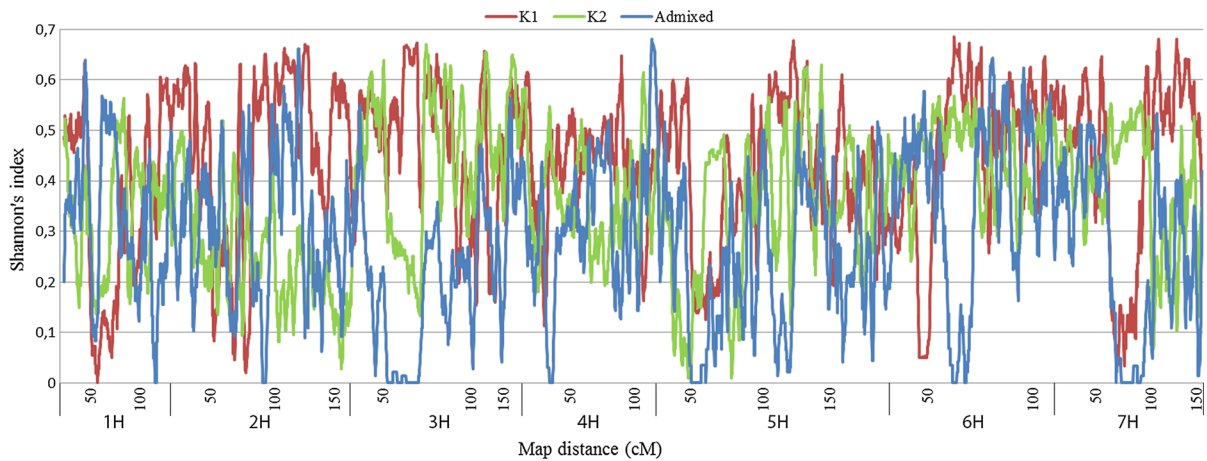


Fig. 2 Shannon's diversity index calculated as rolling means over 20 adjacent loci. The start and end position of each chromosome are indicated with vertical lines at the bottom of the figure

Table 4 Number of intra-chromosomal marker-pairs in the total population and in the different groups

Unlinked marker-pairs refers to a marker-pair distance >50 cM

	Population size	Total pairs	Unlinked pairs	Unlinked pairs (%)
Total population	169	1,795,852	744,332	41
Two-row lines	124	1,464,051	609,353	42
Six-row lines	45	923,896	367,226	40
K1 _{SNP}	109	1,166,044	482,154	41
K2 _{SNP}	47	981,129	393,735	40
Admixed _{SNP}	13	663,021	262,178	40

Table 5 Mean r^2 -values for intra-chromosomal marker-pairs in the whole genome and for each chromosome

Chromosome	No. of SNPs	Total population	Two-rowed lines (n = 124)	Six-rowed lines (n = 45)	K1 _{SNP} (n = 109)	K2 _{SNP} (n = 47)	Admixed _{SNP} (n = 13)
1H	437	0.12	0.05	0.11	0.05	0.11	0.15
2H	768	0.11	0.07	0.12	0.06	0.11	0.23
3H	724	0.07	0.08	0.10	0.08	0.10	0.17
4H	578	0.09	0.07	0.11	0.06	0.10	0.18
5H	1025	0.10	0.10	0.10	0.05	0.10	0.21
6H	726	0.09	0.09	0.14	0.10	0.13	0.17
7H	626	0.09	0.07	0.08	0.07	0.09	0.19
Whole genome	4884	0.10	0.08	0.11	0.07	0.10	0.19

consideration, the SNPs would be just as or even more informative than the SSRs. A higher number of private alleles were found with the SNPs compared to the SSRs (Tables 1, 2). However, considering the different number of markers, the SSRs actually had the highest private allele frequency, which could be

expected since the SNPs are bi-allelic and have a lower mutation rate compared to the SSRs (Martinez-Arias et al. 2001; Li et al. 1981; Kruglyak et al. 1998).

It has earlier been reported that there is strong correlation between these two marker systems in barley (Varshney et al. 2008; Varshney et al. 2010).

Table 6 Interval of the estimated LD decay (cM) in the total population and for the different groups

Chromosome	No. of SNPs	Total population	Two-rowed lines (n = 124)	Six-rowed lines (n = 45)	K1 _{SNP} (n = 109)	K2 _{SNP} (n = 47)	Admixed _{SNP} (n = 13)
1H	437	0–3	6–9	3–6	6–9	3–6	3–6
2H	768	0–3	3–6	3–6	6–9	3–6	0–3
3H	724	7–11	7–11	4–7	7–11	4–7	4–7
4H	578	0–3	3–5	0–3	3–5	0–3	0–3
5H	1025	0–4	0–4	0–4	8–12	4–8	0–4
6H	726	0–3	5–8	0–3	5–8	0–3	0–3
7H	626	0–3	7–10	7–10	7–10	3–7	0–3
Whole genome	4884	0–4	4–8	0–4	8–12	0–4	0–4
Background LD whole genome ^a		0.20	0.16	0.19	0.10	0.19	0.31

^a The 95th percentile of unlinked (above 50 cM) square root transformed r^2 values

This was also demonstrated here with a strong and positive correlation ($r = 0.86$) between the modified Roger's distances based on the SSR data and the simple matching coefficient based on the SNP data. Also the PCoA and structure analysis grouped the lines in a similar way with the two marker systems. However, the PCoA clustering based on the SNP data showed clearer and more distinct groupings than the SSR-based PCoA, but no major difference in the amount of molecular variance explained was found between the two marker systems. Neither did the AMOVA analyses of the structure groups reveal any large differences between the two marker systems (Table S1). However, superimposing the results from the population structure analysis on the results from the PCoA provided a clearer image and higher resolution of the population structure based on the SNPs compared to the SSRs (Fig. 1a, b). That reveals the ability of SNPs to explain the population structure at a more specific level. This was also seen in a study of genetic diversity and population structure of 375 rice varieties (Singh et al. 2013), where a comparison of the SSR and SNP marker systems revealed that at the structure level the SNPs were better at describing genetic relatedness whereas at the diversity level the SSRs showed a better grouping of samples. In contrast, some studies of genetic diversity and population structure in maize report a better estimate of population structure with SSRs compared to SNPs (Yang et al. 2011; Hamblin et al. 2007). The different results between those reports and this study might be due to the different numbers of SNP markers used (<900 vs.

6208) or due to the complexity of the maize genome. According to a theoretical prediction by Laval et al. (2002), $(k - 1)$ times more bi-allelic markers are needed to achieve a comparable accuracy of the genetic distance as a set of SSRs with k alleles. With the average of about 3 alleles per SSR marker in this study, the number of SNPs needed would be $[(3 - 1) \times 48] = 96$, which are about 65 times less compared to the 6208 used. Altogether the results from this study show that the numbers of SNPs used are more than enough to retrieve a comparable accuracy of genetic diversity in barley as the set of SSRs used. In addition, the SNPs seem to provide a higher resolution for the genetic relatedness than obtained with the SSRs.

Diversity and relationships within Nordic spring barley

The average genetic diversity for the Nordic spring barley collection analysed here was 0.514 and 0.359 based on the SSRs and SNPs, respectively. These are similar to the diversity estimates of Nordic breeding lines and cultivars released after 1970 (0.601) reported in an earlier study based on SSRs (Kolodinska Brantestam et al. 2007). In addition a similar result based on SSRs was reported in accessions from Europe (0.593), Eritrea (0.573) and Ethiopia (0.620), whereas the *Hordeum vulgare* subsp. *spontaneum* (K. Koch) and *H. vulgare* accessions from the West Asia North Africa (WANA) region had a higher diversity (0.826 and 0.762, respectively) (Orabi et al. 2007).

To estimate the genetic relatedness within this Nordic spring barley germplasm, the lines were analysed using Bayesian clustering in STRUCTURE, PCoA and AMOVA analyses. The analyses revealed that the population could be differentiated primarily by ear row-type, but also a further separation due to the geographical origin was found. This was also previously reported by Kolodinska Brantestam et al. (2007) in a study of genetic diversity within Nordic barley. Both ear row-type and geographical origin are factors known to cause population structure in barley (Malysheva-Otto et al. 2006; Hamblin et al. 2010; Cockram et al. 2010; Rostoks et al. 2006). The progenitor of cultivated barley is two-rowed and the six-rowed barley was selected shortly after domestication and originated from independent mutations of the *Vrs1* allele (Komatsuda et al. 2007). Moreover, there has been a geographic separation of the two ear row-types in the Nordic countries, where two-rowed barley cultivars have been preferred in the southern and earlier, and six-rowed barley cultivars in the northern region. In this study two groups, K1 and K2, with a clear ancestry and a small group with admixed ancestry was inferred from STRUCTURE. The classification of K1 and K2 corresponded to the two-rowed lines from the southern region and the six-rowed lines, respectively, whereas the lines in the admixed group mainly corresponded to the two-rowed lines from the northern region. Structure corresponding to row-type and growth habit, but also due to breeding program, have earlier been reported of in American barley germplasm (Hamblin et al. 2010). Hamblin et al. (2010) reported that the separation observed based on breeding programs appeared to have been generated by recent breeding history and that local adaptation could have contributed to this structure. Local adaptation, due to differences in day length between the northern and southern region could also be a likely explanation of the geographical separation of the lines observed in this study. No large differences in genetic diversity between the population structure groups or row-types was found in the current study. One reason for this could be the difference in the number of lines within each group. The different ear row-types explained 40% of genetic variation in the population, whereas only an additional 2% was explained by the population structure groups. However, even if

no large differences in genetic diversity could be found between the different population structure groups of the population, differences in diversity could be identified in specific chromosomal regions. In a previous study of European two-rowed spring barley, a lack of diversity was observed on 2H at the region where the *HvCEN* locus are positioned (Comadran et al. 2012; Tondelli et al. 2013). The *HvCEN* locus is associated with late flowering and high yield, and the lack of diversity in this region was explained as a result of fixation of *HvCEN* in European two-rowed spring barley (Tondelli et al. 2013). Unsurprisingly, since early flowering is an important characteristic in Nordic barley, and more so in the northern part of the region than in the southern, no lack of diversity on 2H was evident in this study, neither was it shown in a previous study of Nordic barley (Rajala et al. 2016). Low diversity was seen for the six-rowed lines in K2_{SNP} and the two-rowed lines from the northern parts in the admixed_{SNP} group at certain regions on 2H (83–113 cM), 3H (37–64 cM) and 5H (108–127 cM). In these chromosomal regions are the *Vrs1* allele (2H) determining the row-type and genes related to the photoperiod pathway and circadian clock such as *HvGI*, *HvFT2* (3H) and *VRNH1* (5H), all of them characteristics important in barley breeding for the northern regions (Dunford et al. 2005; Sasani et al. 2009; Komatsuda et al. 2007; Distelfeld et al. 2009; Faure et al. 2007; Ramsay et al. 2011). In a previous study of genetic diversity in European two-rowed spring barley, low PIC values were observed at regions on chromosome 1H (47.8–55.4), 6H (30.2–53.6) and 7H (29.8–47.6), which were postulated to be a result of human selection reflecting targets like malting quality traits (Tondelli et al. 2013). Also in the present study the two-rowed lines in group K1_{SNP} showed a low diversity at 6H (45–55 cM), in contrast to the other population structure groups where the diversity was maintained. On the corresponding region on 7H the diversity was maintained for all groups, however, a prominent low diversity was evident within another genomic region (around 62–88 cM) on chromosome 7H in the K1_{SNP} and the admixed_{SNP} group. This region close to the centromere on 7H has previously been described to contain several important QTLs controlling malt extract, α -amylase activity, diastatic power and β -glucanase (Hayes et al. 2003).

Linkage disequilibrium within Nordic spring barley

Since the population in this study has a clear and strong structure the LD was estimated in both the whole population, without considering the structure, as well as in the subpopulations estimated from the STRUCTURE analysis. The background LD (r^2) for the whole population was 0.20 (Table 6). For the different row-types as well as the population structure groups the background LD was lower (0.10–0.19), except for the admixed_{SNP} group which had a background LD of 0.31. However, the admixed_{SNP} group is a very small group with only 13 lines and with lines from four of the six breeders, and also with a more complex structure. These results are in accordance with the results from a study of LD in elite barley breeding germplasm from the United States by Zhou et al. (2012), where the background LD decreased when the structure was considered, but was still high in more diverse sub-populations with unexplained structure.

In this study there was a slower LD decay found in the two-rowed lines from the southern part, compared to the six-rowed lines and the two-rowed lines from the northern part that may be a result of the focused breeding for higher malting quality in the southern parts. This result is in accordance with an earlier study where a faster LD decay were found in six-rowed compared to two-rowed barley (Pasam et al. 2012). The LD decay in a whole-genome survey for modern two-rowed spring barley cultivars in Europe has been estimated to be at least 10 cM (Kraakman et al. 2004), whereas another study of LD decay in a world-wide spring barley collection reports of LD decay below a critical value of r^2 of 0.2 within a map distance of 7 cM (Pasam et al. (2012), which is in accordance with the results of the two-rowed lines in this study (Table 6).

In the chromosome comparison, a rapid average LD decay was found within all groups on chromosome 4H that might be due to introduction of important traits that occurs during breeding, e.g. *mlo*. In this study the occurrence of the *mlo* locus on 4H is about 50% within the population, which may indicate that there is a targeted recombination due to *mlo* introgression in half of the population that might be contributing to the rapid LD decay.

Conclusion

The results obtained with the two marker systems in this study correlated well and both systems revealed a strong structure based on the row-types, but also on geographical origin. No large differences in genetic diversity between the row-types or population structure groups were observed, however differences in certain chromosomal regions was evident which could reflect different breeding aims for the northern and southern regions. The results from this study help breeders in the Nordic region to understand genetic structure and diversity in their barley breeding material better. This is of importance when planning breeding programs for higher genetic gains.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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