Development and characterization of polymorphic EST based SSR markers in barley (*Hordeum vulgare*)

Won-Sam Jo¹ · Hye-Yeong Kim¹ · Kyung-Min Kim¹

Received: 4 February 2017 / Accepted: 3 June 2017 / Published online: 26 July 2017
© Springer-Verlag GmbH Germany 2017

Abstract In barley, breeding using good genetic characteristics can improve the quality or quantity of crop characters from one generation to the next generation. The development of effective molecular markers in barley is crucial for understanding and analyzing the diversity of useful alleles. In this study, we conducted genetic relationship analysis using expressed sequence tag-simple sequence repeat (EST-SSR) markers for barley identification and assessment of barley cultivar similarity. Seeds from 82 cultivars, including 31 each of naked and hulled barley from the Korea Seed and Variety Service and 20 of malting barley from the RDA-Genebank Information Center, were analyzed in this study. A cDNA library of the cultivar Gwanbori was constructed for use in analysis of genetic relationships, and 58 EST-SSR markers were developed and characterized. In total, 47 SSR markers were employed to analyze polymorphisms. A relationship dendrogram based on the polymorphism data was constructed to compare genetic diversity. We found that the polymorphism information content among the examined cultivars was 0.519, which indicates that there is low genetic diversity among Korean barley cultivars. The results obtained in this study may be useful in preventing redundant investment in new cultivars and in resolving disputes over seed patents. Our approach can be used by companies and government groups to develop different cultivars with distinguishable markers. In addition, the developed markers can be used for quantitative trait locus

analysis to improve both the quantity and the quality of cultivated barley.

Keywords Dendrogram · EST-SSR marker · Genetic similarity · Barley

Introduction

Barley (*Hordeum vulgare* L.) has been cultivated for around 10,000 years and is a crop that adapts well to various environments. It is used as a cereal grain, animal fodder, and in the production of beer and distilled beverages, and ranks in importance with other crops such as rice, wheat, and corn. In terms of morphology and use, barley can be classified into three groups: naked, hulled, and malting barley. Naked barley can easily be extracted from the outer and inner glumes, and is an important crop in South Korea, China, and Japan. Hulled barley, which is extracted from the seed coat with difficulty, is cultivated internationally. Recently, molecular markers have been widely used for mapping genes of interest and for marker-assisted breeding (Gupta and Varshney 2000). Several types of slowly evolving molecular markers have been identified. Among these, however, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphisms (AFLPs) are not suitable for wide screening and assessment of genetic diversity among cereal species. RFLP analysis is not easily scalable for use in high-throughput methods, and RAPD analysis is often not reproducible or transferable between laboratories (Shariflou et al. 2001). Also multiple AFLPs were used to analyze large genetic template to identify the individual bands (Shan et al. 1999).

✉ Kyung-Min Kim
kkm@knu.ac.kr

¹ School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 41566, Korea

Microsatellites are simple repeated motifs that consist of one-to-six base pairs. Using polymerase chain reaction (PCR)-based microsatellites as genetic markers can produce codominant markers. The advantages of microsatellites are high polymorphism rates, high abundance, and broad distribution throughout large genomes (Morgante et al. 2002; Wright and Bentzen 1995). The establishment of an international barley genome project has resulted in the production of genetic maps and molecular markers have been made available to the public. Graner et al. (1991) and Kleinhofs et al. (1993) constructed the first genetic map of barley using RFLP markers, and Ramsay et al. (2000) and Li et al. (2003) later developed microsatellite-based genetic maps of barley using 242 and 127 SSR markers, respectively. Subsequently, Varshney et al. (2007) used 775 simple sequence repeat (SSR) markers to produce a high-density map for barley. Furthermore, a barley mapping population consisting of 96 doubled haploid lines of anther culture origin was developed from the varieties Dicktoo and Kompolti Korai, which represent diverse types with respect to geographical origin and ecological adaptation.

Several molecular marker techniques have been used in mapping, among which those markers with known chromosome location (RFLP, STS, and SSR markers) have been applied to identify linkage groups and for comparative mapping, whereas RAPD and AFLP markers, which have random binding but provide useful information on polymorphism, have been employed to fill in the linkage groups with markers (Karsai et al. 2007). In spring barley, 22 polymorphic markers have been reported, including 13 diversity array technology (DArT)-based SSRs (Fiust et al. 2015). In the Netherlands, Germany, Austria, Sweden, France, and the UK, 48 efficient markers for crop identification are used with 24 crops (Macaulay et al. 2001). EST-SSR markers used to construct a genetic map of barley have been published by Pillen et al. (2000), Thiel et al. (2003), Ramsay et al. (2004), and Varshney et al. (2007). Zhang et al. (2014) developed 49 novel EST-SSRs, and with regard to barley identification research in Korea, an analysis of the relationships among 71 varieties using SSR markers has been carried out by Kwon et al. (2011).

Hordein is one of the distinctive proteins in barley seeds, and the electrophoretic patterns of hordein are key characters in determining the quality of barley (Pedersen and Linde-Laursen 1995). The hordein band pattern revealed by SDS-PAGE has been used to distinguish seeds of the barley varieties Alchanbori, Iljinbori, Keunabori, Millyanggeotbori, Neulssalbori, Chalbori, and Sinhobori (So et al. 2004). Nandha and Singh (2014) reported a comparative assessment of genetic diversity between wild and cultivated barley using gSSR and EST-SSR markers,

whereas Bungartz et al. (2016) reported a CAPS (cleaved amplified polymorphic sequence) marker set using SNP (single-nucleotide polymorphism) genotyping. Furthermore, high-resolution melting technology has been used to genotype 55 InDel (insertion and deletion) markers (Zhou et al. 2015) and marker enrichment in the target region has been carried out by amplified barley EST- and rice homologous gene-based searching (Jia et al. 2016).

Rudd (2003) reported a useful type of PCR-based microsatellite, expressed sequence tag-simple sequence repeat (EST-SSR), which is applicable for use in gene discovery, genome annotation, and comparative genomics. Using raw data, ESTs within ESAP plus can be uploaded via a submission page of a web interface (Ponyared et al. 2016). Zhang et al. (2014) developed 49 novel EST-SSRs and confirmed 20 genomic SSRs for 80 Tibetan annual wild barley and 16 cultivated barley accessions. It has, however, proven difficult to establish DNA profile databases for other varieties of barley, because the precision of PCR amplification is low. It is, therefore, necessary to develop a diversity of efficient molecular markers for analysis and identification of useful alleles associated with important traits. To establish a standardized database in barley, additional research is needed using precision equipment. Hence, this study was conducted to examine the possibility of discriminating among domestic varieties using EST-SSR markers and to determine whether these markers could be used to resolve disputes regarding patented seeds.

Materials and methods

Plant materials

Seeds of 82 barely varieties (31 naked and 31 hulled barleys from the Korea Seed and Variety Service and 20 malting barleys from the RDA-Genebank Information Center) were used in this study (Table 1).

Extraction of genomic DNA and total RNA

DNA was extracted from the barley seeds using a Nucleospin® Plant Kit (Macherey-Nagel Cat. 740 570.50). The concentration of the extracted DNA was measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and adjusted to $20 \text{ ng } \mu\text{L}^{-1}$ for storage at -20°C . Total RNA was extracted using an RNeasy Plant Mini Kit (QIAGEN, Germany). The extracted RNA was suspended in $30 \mu\text{L}$ of RNase-free water, and then stored at -80°C in a deep freezer.

Table 1 List of the 82 studied barley varieties received from the National Institute of Crop Science

No.	Naked barley	No.	Hulled barley	No.	Malting barley
1	Ganghossalbori	1	Gangbori	1	Gwangmaekbori
2	Gwanghwalssalbori	2	Geongangbori	2	Namhyangbori
3	Ginssalbori	3	Gwanganbori	3	Dajinbori
4	Namhossalbori	4	Nangnyeongbori	4	Dahobori
5	Naehanssalbori	5	Dahyangbori	5	Danwonbori
6	Neulssalbori	6	Daebaekbori	6	Daeabori
7	Dasongssalbori	7	Daeyeonbori	7	Daeyeongbori
8	Dapungssalbori	8	Daejinbori	8	Dusan8ho
9	Daehossalbori	9	Mirakbori	9	Dusan29ho
10	Donghanssalbori	10	Millyanggeotbori	10	Maekyangbori
11	Donghossalbori	11	Samgwangchalbori	11	Baekobori
12	Duwonchalssalbori	12	Sangnokbori	12	Sacheon6ho
13	Baekdong	13	Saegangbori	13	Samdobori
14	Saeneulssalbori	14	Saealbori	14	Sinhobori
15	Saessalbori	15	Saeolbori	15	Oreumbori
16	Saechalssalbori	16	Seodunchalbori	16	Iljinbori
17	Saehanchalssalbori	17	Albori	17	Jejubori
18	Songhak	18	Alchanbori	18	Jingwangbori
19	Olssalbori	19	Yeongyangbori	19	Jinnyangbori
20	Jasujeongchalssalbori	20	Owolbori	20	Hopumbori
21	Jaegangssalbori	21	Olbori		
22	Jaanssalbori	22	Chalbori		
23	Jinmichapssalbori	23	Keunalbori		
24	Jinjuchalssalbori	24	Keunalbori1ho		
25	Chalssalbori	25	Tapgolbori		
26	Cheonghossalbori	26	Taegangbori		
27	Chunchussalbori	27	Taepyeongbori		
28	Pungsanchalssalbori	28	Paldobori		
29	Hobanssalbori	29	Hyedangbori		
30	Huinssalbori	30	Hyemibori		
31	Huinchalssalbori	31	Hwanggeumchalbori		

Construction of a cDNA library

The cDNA library was constructed using a cDNA Library Kit (TaKaRa code 6119 and 6130, TaKaRa, Japan). One microliter of PCR product was loaded on a 1% agarose gel and the results were confirmed on a UV transilluminator. To remove PCR enzymes and other impurities, a PCR Purification Kit (QIAGEN, Germany) was used and the cDNA was ligated to a pGEM-T Easy Vector (Promega, USA). To ligate the cDNA to the plasmid vector, 50 ng μL^{-1} of pGEM-T Easy Vector was mixed with the cDNA PCR product, along with 5 μL of $2\times$ rapid ligation buffer and 1 μL of T4 DNA ligase (3 units μL^{-1}). Nuclease-free water was added to give a total volume of 10 μL , and then,

the mixture was maintained at 4 °C for 12 h. Five microliters of ligation product were added to *Escherichia coli* DH 5 α competent cells, which were maintained on ice for 30 min and then at 42 °C for 1 min. After treatment on ice for 2 min, 250 μL of LB broth (10 g bacto tryptone, 5 g bacto yeast extract, and 5 g NaCl) was added and the cells were cultured at 37 °C and 180 rpm for 1 h. The cell culture was then spread on LB solid medium (containing 100 ppm ampicillin, 0.5 mM IPTG, and 80 $\mu\text{g mL}^{-1}$ X-Gal) and cultured at 37 °C for 12 h. After culturing, white colonies were selected and re-cultured in 5 mL of LB broth at 37 °C for 16 h. The broth was centrifuged for 10 min and plasmid DNA from the *E. coli* was extracted using a QIAprep Spin Mini Prep Kit (QIAGEN, Germany).

Restriction enzyme treatment

One microliter of *Eco*RI restriction enzyme (TaKaRa, Japan) was incubated with 3 µL plasmid DNA, 2 µL of 10× buffer (H), and 14 µL of nuclease-free water at 37 °C for 1 h. The mixture was then maintained on ice for 10 min to prevent a chain reaction of plasmid DNA cutting, and subsequently loaded on a 1% agarose gel containing 1 µL of ethidium bromide. The cut cDNA was confirmed on a UV transilluminator.

Polymorphism analysis by PCR

Polymerase chain reaction was conducted using a UNO II Thermocycler (Biometra, Germany). As a template, 25 ng of barley genomic DNA was added to 2.5 mM 10× buffer (500 mM KCl; 100 mM Tris-HCl, pH 8.3; and 15 mM MgCl₂), 20 pmol dNTP mixture, 20 pmol SSR markers, and 1 unit of Taq DNA polymerase. Initial denaturation was started at 94 °C for 5 min, and denaturation was at 94 °C for 30 s. The annealing temperature ranged from 47 to 60 °C for 30 s, and extension was at 72 °C for 1 min. The final extension temperature was 72 °C for 5 min. After the PCR was complete, 5 µL of product was loaded into a QIAxcel capillary gel electrophoresis system (QIAGEN, Germany) for genotyping.

Analysis of genetic similarity

Amplified PCR products were genotyped using the QIAxcel system (QIAGEN, Germany). All types were scored with regard to the presence or absence of the corresponding bands among the genotypes. The scores “1” and “0” indicate the presence and absence, respectively, of the bands for each allele of the SSR markers. In the several cases in which more than two alleles were observed in a given genotype, we used the data for these parameters previously determined by Aggarwal et al. (2007). The polymorphism information content (PIC) was calculated via the formula established by Powell et al. (1996) and Smith et al. (1997):

$$PIC = \sum_{j=1}^n f_i^2$$

where f_i is the frequency of the j th allele and the summation extends over n alleles.

Genetic similarity was estimated from binary matrices using Jaccard's coefficient (Sneath and Sokal 1973). A cluster analysis was conducted on the basis of the binary data using NTSYS-PC version 2.10b (Rohlf 2000) with the unweighted pair group method using the arithmetic averages (UPGMA). The simple matching coefficient (Sneath

and Sokal 1973) of similarity between entries was calculated for morphological analysis, and UPGMA methodology was used for dendrogram construction, as has been previously described by Bernet et al. (2003).

Results

Construction of a cDNA library, restriction enzyme treatment, and development of EST-SSR markers

Total RNA from seeds of a representative cultivar, Gwanbori, was used to construct cDNA libraries. The total of 128 cDNA libraries were ligated with pGEM-T vectors (Promega, USA) for transformation of *E. coli*. The average length of the cDNA libraries is 98 bp (Table 2). Of the 58 identified EST-SSR markers, most contain mono-nucleotide repeat motifs (35, 30.3%), followed by hexa- (13, 22.4%), penta- (9, 15.5%), and tri-nucleotide repeat motifs (1, 1.7%). The 58 forward and reverse primer pairs used as EST-SSR markers were designed using a web program and the SSR regions were identified (Table 3); 15 of these primers were confirmed as EST-SSR. To analyze their polymorphisms, PCR was conducted using 82 varieties of barley and 62 primers, including 47 SSR primers and the 15 developed EST-SSR markers (Table 4). The amplified products were analyzed using a QIAxcel capillary gel electrophoresis system (QIAGEN, Germany) (Fig. 1).

Establishment of a DNA profile database

The similarity of the barley cultivars was evaluated with respect to PIC and PCR band type. The PIC values, a reflection of allele diversity and frequency among the varieties, were not uniformly high for the SSR loci tested. The PIC values ranged from 0.286 to 0.952, with a mean of

Table 2 Summarization of EST-SSR search results

Searching items	Numbers
Total number of sequences examined	128
Total size of examined sequences (bp)	12,544
Total number of identified SSRs	58
Number of sequences containing SSR	52
Number of sequences containing more than 1 SSR	3
Mono-nucleotide	35
Di-nucleotide	0
Tri-nucleotide	1
Tetra-nucleotide	0
Penta-nucleotide	9
Hexa-nucleotide	13

Table 3 List of EST-SSR markers developed from the sequencing data

No.	Primer	Primer (5'–3')	Primer (3'–5')	Repeat motif	Expected size
1	GK0009	AACTGCGCGCTAACCTAGAA	TAGACCTGCAGGCTCGAGTT	A(15)	157
2	GK0033	TATCCAGTGCGAATACCTCGG	GCTTCGGGGAGTTGAAAATAAG	T(15)	257
3	GK0045	TAGACCTGCAGGCTCGAGTT	GTCAACGAGTCGGGTGTTT	T(14)	136
4	GK0047	GGTACCACGCGTGATCTA	TAGGTCCATGAGCAGGCAAG	T(15)	152
5	GK0067	ACCTGCAGGCTCGAGTTT	AAACTGCCTGCTGAATCCAT	T(15)	100
6	GK0096	CGGGGAGTTGAAAATAAGCA	TAGACCTGCAGGCTCGAGTT	A(13)	155
7	GK0116	AGACCTGATGCGGACCTACTTA	TTAAACTGCCTGCTGAATCCAT	T(15)	160
8	GK0119	ACCTGGCGAACTGAAACATC	GCCGACACTGACACTGAGAG	T(15)	115
9	GK0126	TGCTTTCGGCTACTGGACTT	CCGGAGATTCCCAAATAGGT	T(15)	258
10	GK0131	TAGACCTGCAGGCTCGAGTT	AAGTCCAGTAGCCGAAAGCA	T(15)	133
11	GK0155	TGGGAATCTCCGGATCTATG	ACAACCTGGCGAACTGAAAC	T(15)	177
12	GK0156	CCCAAGTCCCCTGGAAAG	TAGACCTGCAGGCTCGAGTT	A(15)	221
13	GK0168	CCTCAGCCTACGGGGTATTAG	CGGGGAGTTGAAAATAAGCAT	T(13)	303
14	GK0189	CCCAAGTCCCCTGGAAAG	TAGACCTGCAGGCTCGAGTT	A(14)	213
15	GK0191	TAGACCTGCAGGCTCGAGTT	TCACGAATCAGAGTGCCAAG	T(14)	188
16	GK0225	TAGACCTGCAGGCTCGAGTT	GTCAACGAGTCGGGTGTTT	T(14)	135
17	GK0233	AAGTCCAGTAGCCGAAAGCA	CATGAGCAGGCAAGAGACAA	T(15)	243
18	GK0240	ACGGTTGCTAATACCCGCTAG	GCTTTTGCTTTCTTTCTCTG	A(14)	319
19	GK0268	AAAAGGGCGAATACAACCA	GCGTGGTACCATGGTCTAGAGT	A(11)	150
20	GK0276	CTAGAAGCAGCCACCCTTTA	TTTTTCGTTACTCAAGCCGA	GAAGCG(2)	204
21	GK0300	TAGACCTGCAGGCTCGAGTT	GTCAACGAGTCGGGTGTTT	T(14)	136
22	GK0329	TAGACCTGCAGGCTCGAGTT	CCCAAGTCCCCTGGAAAG	T(14)	213
23	GK0334	ACTACCCGCTGAGTTTAAGCAT	TGTAAGTTTCTTCTCCTCCGCT	A(13)	172
24	GK0337	AGAAACCCTAATCAGCCACGTA	TGTTGTACTCATCGACATGCAA	ATGTG(2), TGATG(2)	180
25	GK0337	TGCATGTCGATGAGTACAACAA	CCCTTATCTTTCTCCTACCGTCT	TTACT(2)	326
26	GK0359	ATCAGGTCTCCAAGGTGAACA	ACCCCTAACCACAACCTCATCC	A(15)	165
27	GK0375	GTCAACGAGTCGGGTGTTT	TAGACCTGCAGGCTCGAGTT	A(15)	137
28	GK0385	ATTGAGATCGGAAAGAACACCA	CCTAGTATCCATCGTTTACGGC	ACACTG(2)	131
29	GK0389	TAGACCTGCAGGCTCGAGTT	CAAGCTGGAGTACGGTAGGG	TCAGTG(2)	152
30	GK0396	GACCTACTTAGTCGACTCTAGACCA	GGCAGGGTCCGAGGTATT	T(14)	106
31	GK0420	TAGACCTGCAGGCTCGAGTT	AAAATCCGGAGGACCGAGTA	T(16)	167
32	GK0439	CGGGGAGTTGAAAATAAGCA	TCCCTTGCCTACATTGTTC	A(14)	220
33	GK0451	AGTAGCCGAAAGCATCACTAGC	TGTATTTAGCCTTGACGGAGT	A(15)	398
34	GK0451	GAGAGACCGATAGCGAACAAGT	TATGCTTATTTTCAACTCCCCG	A(15)	191
35	GK0462	TACGCCATGCTAGATCGTTG	TAGACCTGCAGGCTCGAGTT	A(14)	174
36	GK0463	CTAAGATGTTTCAGTTCGCCAG	GGGAGTACGTTTCGAAGAAT	T(15)	169
37	GK0468	CACCGCAAGCCACCTACTTA	ACAACCTGGCGAACTGAAAC	T(13)	151
38	GK0472	TAGACCTGCAGGCTCGAGTT	AAACTGCCTGCTGAATCCAT	T(15)	103
39	GK0475	GAGAAGGAAGGACGCTTTCA	GGGAGTACGTTTCGAAGAAT	A(14)	204
40	GK0476	TAGACCTGCAGGCTCGAGTT	CGGGGAGTTGAAAATAAGCA	T(14)	156
41	GK0480	AATGATAGGAAGAGCCGACATC	CAGAAATCTCGTGTGGAACAAA	TTCGTA(2)	218
42	GK0487	CTACCGTTGGTGTTAAAGGGAG	TGTATAGAGAAGGATTTCCCGC	TGGGT(2)	162
43	GK0489	CACTACCGCTGAGAGACCTTTT	TCTGGGTGTTAGGGCATATTT	AGGGAA(2)	322
44	GK0489	TGCACACGATTAGTCCTTTACG	GGCAACACCTTGACTCCT	GAG(2)	278
45	GK0515	TTCCAGGTTAATAGGGAGGAA	CCAATTTGAAGTTGTTGATGTTT	ATAAAT(2)	102
46	GK0517	AATGATAGGAAGAGCCGACATC	CAGAAATCTCGTGTGGAACAAA	TTCGTA(2)	218
47	GK0560	ATTTTACTTATTCGGTGGGTCG	AATGATAGGAAGAGCCGACATC	TACGAA(2)	388
48	GK0572	AAGCTGTGGGATGTCAAAATG	TCTTACCCCTTCTTACCCTGA	GAAGCG(2)	353

Table 3 continued

No.	Primer	Primer (5'–3')	Primer (3'–5')	Repeat motif	Expected size
49	GK0576	AGTAGCCGAAAGCATCACTAGC	ACAATCGCCCTATTAAGACTCG	GGAAC(2)	332
50	GK0580	TGGGTGAACAATCCAACACTT	TAACGCAGGTGTCCTAAGATGA	TTCGTA(2)	290
51	GK0621	AGTAGCCGAAAGCATCACTAGC	ACAATCGCCCTATTAAGACTCG	GGAAC(2)	332
52	GK0630	GCAGCAGTTCTTCCATACCAAC	AGAGACGAAAGTCGGCCATAGT	ACCCA(2)	384
53	GK0674	TCTGCTTGTTGCAATAGTTAGA	ACCTGCAGGCTCCTTAATGC	AGAAT(2)	122
54	GK0683	AAGCTGTGGGATGTCAAAATG	TCTTACCCCTTCTTACCCTGA	GAAGCG(2)	353
55	GK0695	CCGGTCATGTTTCTTGGATT	ACCTGCAGGCTCCTTAATGC	ATTGA(2)	292
56	GK0712	AGTAGCCGAAAGCATCACTAGC	ACAATCGCCCTATTAAGACTCG	GGAAC(2)	332
57	GK0727	TAACGCAGGTGTCCTAAGATGA	TGGGTGAACAATCCAACACTT	TACGAA(2)	290
58	GK0735	TGGTGAGGATGTTGTTGTGAC	ACGCTCTTCGTGTTTCGAGAT	CTTGAG(2)	349

0.519, and the number of alleles ranged from 2 to 5 with a mean of 3.2.

Highly informative SSR markers ($PIC \leq 0.7$), such as Bmag0211, EBmac0711, HVM49, Bmag0378, P9, GMS027, S19, P61, GK0240, GK0489-1, and GK0576, may be used for variety identification and genetic assessment of barley germplasms. The EST-SSR primer pairs used for genetic diversity analysis, the number of alleles for each EST-SSR locus, and the PIC values are shown in Table 5.

Dendrogram using EST-SSR markers

A dendrogram was constructed from the 15 EST-SSR and 47 SSR markers using the NTsyspc2.21 program for genetic relationship analysis (Fig. 2). In the PCR analysis of the 82 cultivars using 62 selected markers, two-to-five amplified bands were clearly distinguishable in each cultivar. The 47 SSR markers were divided into three major group with a similarity level of 0.67 (Fig. 2). Malting barley-type varieties were grouped in cluster I, and were discriminated by SSR marker genotypes. Notably, only two varieties, Duwonchapssalbori and Pungsanchalssalbori, were not grouped with the other naked barley varieties (Fig. 2). Clusters II and III contained 30 naked and 30 hulled-type barley varieties, respectively. The phenogram shows the close genetic similarity (similarity level of 0.8) between the naked and hulled barley groups.

Discussion

Fifty-eight candidate EST-SSR markers were designed from sequence data derived from 380 plasmid DNA samples. In a PCR analysis of 82 cultivars using 62 selected SSR and EST-SSR markers, distinctive amplified bands

were detectable in each cultivar, and a dendrogram was constructed from the PCR data to identify the barley cultivars. The average PIC of the 82 barley cultivars is 0.519, indicating that there is low genetic diversity among these cultivars. The dendrogram shows that naked, hulled, and malting barley cultivars generally clustered in their respective groups. These results are clearer than the results obtained from a dendrogram constructed using 22 morphological markers. The similarities among the 82 cultivated barleys can thus be fully characterized using information from PCR-derived markers.

In the absence of full sequence data from barley cultivars in Korea, we developed fixed Korean barley EST-SSR markers in the present study by transformation of *E. coli* with vectors containing cDNA libraries. Sequence data were obtained from Gwanganbori, which is a typical Korean barley that has similarities with other cultivars of Korean barley. Future studies should consider investigating the classification of Korean barley cultivars using SSR or EST-SSR markers derived European barley cultivars. Previous studies have examined the development of EST-SSR in barley using the GrainGenes database (<http://wheat.pw.usda.gov/GG2/>), and in recent years, a number of studies have also reported the use of these markers in various grains, including wheat, rice, maize, and barley. EST-SSR markers are useful for various purposes, particularly in breeding (Gupta and Varshney 2000).

The 775 genomic DNA-derived SSR marker loci used by Varshney et al. (2007) to produce a high-density map for barley had a higher polymorphism information content value compared to the EST/gene-derived SSR loci. Such a high-density consensus SSR map provides barley molecular breeding programs with a better choice regarding the quality of markers and a higher probability of polymorphic markers in an important chromosomal interval. Several molecular marker techniques have been used in mapping, among which the markers with known chromosome

Table 4 List of SSR markers used for genetic characterization in barley

No.	Marker	Primer (5'–3')	Primer (3'–5')	Repeat motif	Expected size
1	Bmac0134	CCAACTGAGTCGATCTCG	CTTCGTTGCTTCTCTACCTT	AC(28)	148
2	Bmac0209	CTAGCAACTTCCCAACCGAC	ATGCCTGTGTGTGGACCAT	AC(13)	176
3	Bmag0211	ATTCATCGATCTTGTATTAGTCC	ACATCATGTGCGATCAAAGC	CT(16)	174
4	Bmag0323	TTTGTGACATCTCAAGAACAC	TTTGTGACATCTCAAGAACAC	CT(24)	158
5	Bmag0337	ACAAAGAGGGAGTAGTACGC	GACCCATGATATATGAAGATCA	AG(22)	145
6	EBmac0711	CAAAAGCAAAAATCATGAGA	CTAGGTGTGATGAGGGTTTC	–	205
7	Bmag0353	ACTAGTACCCACTATGCACGA	ACGTTCAATAAAATCACAACTG	AG(21)	119
8	HVM49	CTCTATAGGCACGAAAAATTCC	TTGCACATATCTCTCTGTCACA	CA(12)	105
9	HVM40	CGATTCCCTTTTCCAC	ATTCTCCGCCGTCCACTC	GA(6)GT(4)GA(7)	160
10	HVM67	GTCGGGTCCATTGCTCT	CCGGTACCCAGTGACGAC	GA(11)	116
11	HVMLOE	CTTCCATGTACCTACAGC	CGAACTGGTATTCCAAGG	CTT(6)	230
12	EBmac0764	AGAATCAAGATCGACCAAAC	AAAAACATGAACCGATGAA	–	124
13	Bmac0018	GTCCTTTACGCATGAACCGT	ACATACGCCAGACTCGTGTG	AC(11)	138
14	Bmag0382	TGAAACCCATAGAGAGTGAGA	TCAAAAGTTTCGTTCCAAATA	AG(4)AAAG(7)	109
15	EBmac0415	GAAACCCATCATAGCAGC	AAACAGCAGCAAGAGGAG	–	238
16	EBmac0603	ACCGAACTAAATGAACTACTTCG	TGCAAACTGTGCTATTAAGGG	–	149
17	EBmac0871	TGCCTCTGTTGTGTTATTGT	CCCCAAGTGAACATTGAC	–	180
18	HVM09	CTTCGACACCATCACCCAG	ACCAAAATCGCATCGAACAT	TCT(5)	221
19	HVM74	AGGAAGTCATTGCGTGAG	TGATCAAGAATGATAACATGG	GA(13)	163
20	Bmag0217	AATGCTCAAATATCTATCATGAA	GGGGCTGTCACAAGTATATAG	AG(19)	196
21	Bmag0223	TTAGTCACCCTCAACGGT	CCCCTAACTGCTGTGATG	AG(16)	127
22	Bmag0378	CTTTTGTTCCTCGTAGCATCTA	ATCCAACATATAGTAGCAAAGCC	AG(14)	147
23	P181	GTCGTCTCCCTCCCTTCA	CATTGCCAGCACTGTTTC	GAGAG(4)	227
24	P184	CCTACCAAACAACGGAATA	CAGCCAGAAGGTCTACGA	TGC(9)	276
25	P34	GGCGAGGAACTGTTGTTG	GATCGGCTTCATCGTCTACT	CTTC(6)	252
26	P101	CCCCGTATAAAACACCCA	GGCAGAACTTCAGCACCC	ATC(12)	245
27	P30	ACTGCCACTCCATTTAGG	CTGTCTGAGGCTTGCTTT	ATGT(12)	241
28	P83	CTCGGCAAAACAGAGGACA	TTGTAGCAGCGGATGGTC	AAGAA(4)	278
29	P90	CGCAAGCCACAGAGCACA	TCCGTCCGTTTCGTCCATC	GAT(7)	177
30	P9	ATCACAAACAGCCACTGTCCTA	GTGGTGAACCTTGCCCTTG	AC(11)	111
31	P45	CCCACAACACCAACAAAC	GCCCGTAGAATGAACAAGTA	GGTT(5)	229
32	HVBKASI	ATTGGCGTGACCGATATTTATGTTCA	CAAAACTGCAGCTAAGCAGGGGAACA	C(10)	197
33	HVDHN7	TTAGGGCTACGGTTCAGATGTT	ACGTTGTTCTTCGCTGCTG	AAC(5)	177
34	P32	GCAGAATGGCAGAAACAG	CAAGAATGAGCGAAAAGGT	GATG(6)	233
35	S29	AGAATCAAGATCGACCAAAC	AAAAACATGAACCGATGAA	ATAC(13)	140
36	GMS006	TGACCAGTAGGGGCAGTTTC	TTCTTCTCCCTCCCCAC	GA(2)ATAGA(19)	154
37	GMS027	CTTTTCTTTGACGATGCACC	TGAGTTTGTGAGAACTGGATGG	GT(5)CT(2)GT(27)	154
38	P152	ACCAAGCCCACGAGTAGCA	CGACCCGAGGACGACAGAT	CT(11)	251
39	P121	CCCAGGAATAAGAACAGACAC	CACCGCCTAATAGCAACAA	TACAT(4)	287
40	P106	CGAGCCGTTGCTTAGGTC	TCTACTGCCAGGGCGTGA	CTG(8)	206
41	S19	CCCTAGCCTTCCTTGAAG	TTACTCAGCAATGGCACTAG	AG(19)	135
42	P61	CAAATGGAGCCAAGCAAC	CCATCCTTGACGCACATC	GCA(8)	235
43	GMS021	TTAGTCACCCTCAACGGT	CCCCTAACTGCTGTGATG	GA(17)GA(7)	169
44	GMS046	CTTTTGTTCCTCGTAGCATCTA	ATCCAACATATAGTAGCAAAGCC	GA(13)	156
45	P53	AGGGAAAGAAATCCTAAC	TTGACTTGCTTATACACCT	CCAA(5)	224
46	P150	TAAGTAGGTTTGAGGAAGGGAA	CAACATAGACAAGGTGCTGGA	GAGC(5)	265
47	HVCMA	GCCTCGGTTTGACATATAAAG	GTAAGCAAATGTTGAGCAACG	AT(9)	141

Table 4 continued

No.	Marker	Primer (5'–3')	Primer (3'–5')	Repeat motif	Expected size
48	GK0240	ACGGTTGCTAATACCCCGTAG	GCTTTTGCTTTCTTTTCCTCTG	A(14)	319
49	GK0276	CTAGAAGCAGCCACCCTTTA	TTTTCGTTACTCAAGCCGA	GAAGCG(2)	204
50	GK0337	AGAAACCCTAATCAGCCACGTA	TGTTGTACTCATCGACATGCAA	ATGTG(2), TGATG(2)	180
51	GK0385	ATTGAGATCGGAAAGAACACCA	CCTAGTATCCATCGTTTACGGC	AACTG(2)	131
52	GK0487	CTACCGTTGGTGTTAAAGGGAG	TGTATAGAGAAGGATTTCCCGC	TGGGT(2)	162
53	GK0489-1	CACTACCGCTGAGAGACCTTTT	TCTGGGTTGTTAGGGCATATTT	AGGGAA(2)	322
54	GK0489-2	TGCACACGATTAGTCCTTTACG	GGCAACACCCTTGACTCCT	GAG(2)	278
55	GK0517	AATGATAGGAAGAGCCGACATC	AAGCTGTGGGATGTCAAAATG	TTCGTA(2)	218
56	GK0560	ATTTTACTTATTCGTGGGTCG	AAGCTGTGGGATGTCAAAATG	TACGAA(2)	388
57	GK0621	AAGCTGTGGGATGTCAAAATG	AAGCTGTGGGATGTCAAAATG	GGAAC(2)	332
58	GK0630	GCAGCAGTTCTTCATACCAAC	AGAGACGAAAGTCGGCCATAGT	CTTGAG(2)	349
59	GK0576	AGTAGCCGAAAGCATCACTAGC	ACAATCGCCCTATTAAGACTCG	GGAAC(2)	332
60	GK0695	CCGGTCATGTTTCTTGATT	ACCTGCAGGCTCCTTAATGC	ATTGA(2)	292
61	GK0712	AGTAGCCGAAAGCATCACTAGC	ACAATCGCCCTATTAAGACTCG	GGAAC(2)	332
62	GK0683	AAGCTGTGGGATGTCAAAATG	TCTCTACCCCTTCTTACCCTGA	GAAGCG(2)	353

Fig. 1 Amplified band patterns of 82 barley cultivar DNAs using EST-SSR marker by QIAxcel capillary electrophoresis

location (RFLP, STS, and SSR) have been applied to identify linkage groups and for comparative mapping, whereas RAPD and AFLP markers have been employed to fill in the linkage groups with markers (Karsai et al. 2007). DaRT and SNP genotyping require special tools, and

detection of SSR polymorphisms requires time-consuming polyacrylamide electrophoresis. Furthermore, many markers have been mapped in different populations, such that their genetic positions are inconsistent (Fiust et al. 2015). Recently, InDel (insertion and deletion) markers have

Table 5 Characteristics of developed SSR markers

No.	Marker						Total	PIC	1-PIC	No. alleles
1	Bmac0134	38	29	11	4		82	0.360	0.640	4
2	Bmac0209	40	31	5	5	1	82	0.388	0.612	5
3	Bmag0211	75	6	1			82	0.842	0.158	3
4	Bmag0323	60	20	2			82	0.595	0.405	3
5	Bmag0337	39	35	6	1	1	82	0.414	0.586	5
6	EBmac0711	80	2				82	0.952	0.048	2
7	Bmag0353	50	24	8			82	0.467	0.533	3
8	HVM49	67	15				82	0.701	0.299	2
9	HVM40	35	29	12	6		82	0.334	0.666	4
10	HVM67	31	25	23	3		82	0.316	0.684	4
11	HVMLOE	61	14	7			82	0.590	0.410	3
12	EBmac0764	41	41				82	0.500	0.500	2
13	Bmac0018	46	25	10	1		82	0.423	0.577	4
14	Bmag0382	41	36	3	2		82	0.445	0.555	4
15	EBmac0415	49	26	6	1		82	0.463	0.537	4
16	EBmac0603	44	36	2			82	0.481	0.519	3
17	EBmac0871	51	23	6	2		82	0.471	0.529	4
18	HVM09	62	19	1			82	0.626	0.374	3
19	HVM74	31	22	20	9		82	0.286	0.714	4
20	Bmag0217	53	19	10			82	0.486	0.514	3
21	Bmag0223	31	26	24	1		82	0.329	0.671	4
22	Bmag0378	73	7	1	1		82	0.800	0.200	4
23	P181	57	16	9			82	0.533	0.467	3
24	P184	35	33	13	1		82	0.369	0.631	4
25	P34	41	35	5	1		82	0.436	0.564	4
26	P101	41	23	13	3	2	82	0.356	0.644	5
27	P30	50	18	12	1	1	82	0.442	0.558	5
28	P83	46	25	11			82	0.426	0.574	3
29	P90	47	35				82	0.511	0.489	2
30	P9	69	11	2			82	0.727	0.273	3
31	P45	60	18	2	2		82	0.585	0.415	4
32	HVBKASI	41	32	9			82	0.414	0.586	3
33	HVDHN7	49	25	6	2		82	0.456	0.544	4
34	P32	41	35	5	1		82	0.436	0.564	4
35	S29	37	32	13			82	0.381	0.619	3
36	GMS006	44	33	5			82	0.454	0.546	3
37	GMS027	68	11	3			82	0.707	0.293	3
38	P152	55	25	2			82	0.543	0.457	3
39	P121	53	29				82	0.543	0.457	2
40	P106	40	35	7			82	0.427	0.573	3
41	S19	74	8				82	0.824	0.176	2
42	P61	67	15				82	0.701	0.299	2
43	GMS021	53	29				82	0.543	0.457	2
44	GMS046	46	25	11			82	0.426	0.574	3
45	P53	42	40				82	0.500	0.500	2
46	P150	40	27	9	6		82	0.364	0.636	4
47	HVCMA	38	32	12			82	0.388	0.612	3

Table 5 continued

No.	Marker					Total	PIC	1-PIC	No. alleles
48	GK0240	69	11	1	1	82	0.726	0.274	4
49	GK0276	34	32	16		82	0.362	0.638	3
50	GK0337	40	32	9	1	82	0.402	0.598	4
51	GK0385	38	37	7		82	0.426	0.574	3
52	GK0487	44	38			82	0.503	0.497	2
53	GK0489-1	76	5	1		82	0.863	0.137	3
54	GK0489-2	67	8	7		82	0.684	0.316	3
55	GK0517	64	11	7		82	0.634	0.366	3
56	GK0560	50	13	12	7	82	0.426	0.574	4
57	GK0621	39	33	10		82	0.403	0.597	3
58	GK0630	62	20			82	0.631	0.369	2
59	GK0576	70	12			82	0.750	0.250	2
60	GK0695	60	22			82	0.607	0.393	2
61	GK0712	60	17	5		82	0.582	0.418	3
62	GK0683	55	21	6		82	0.521	0.479	3
						Average	0.519	0.481	3.2

PIC polymorphism information content

become popular in genetic map construction and map-based cloning (Zhou et al. 2015).

PCR-based CAPS marker sets have the advantage of being applicable for use in high-throughput SNP genotyping, and these markers showed the proof of concept for the development and utility of a newer cost-effective genomic tool kit to analyze broader genetic resources of barley worldwide (Bungartz et al. 2016).

Cluster analysis discriminated all 47 accessions, and classified wild and cultivated genotypes into two distinct groups according to their geographic origin. Our analysis indicated that gSSRs are more informative than EST-based SSRs (Nandha and Singh 2014). More specifically, microsatellite markers are widely used in marker-assisted selection programs to develop cultivars that are durably resistant against specific diseases (Miah et al. 2013). Gailing et al. (2013) constructed genetic linkage maps of *Quercus robur* using EST-SSR markers and performed quantitative trait locus (QTL) analysis.

One study of Tibetan barley (Zhang et al. 2014) used EST-SSR markers to assess the differences in PIC between Tibetan annual barley and cultivated barley for the purpose of germplasm appraisal, and genetic diversity and population structure analyses. These EST-SSR markers have potential for application in germplasm appraisal and genetic diversity and population structure analyses, thereby facilitating marker-assisted breeding and crop improvement in barley. With the advent of high-throughput sequencing technology, huge amounts

of EST sequence data have been generated and are now accessible from many public databases. However, SSR marker identification from a large in-house or public EST collection requires a computational pipeline that makes use of several standard bioinformatic tools to design high-quality EST-SSR primers. Some of these computational tools are not user-friendly and must be tightly integrated with reference genomic databases (Ponyared et al. 2016).

In the present study, we developed EST-SSR markers that can be used in the construction of barley maps, in breeding, and even in allocating investments when developing new cultivars. Furthermore, they will be helpful in resolving patent disputes.

Conclusion

In this study, we analyzed the seeds from 82 cultivars of barley, including 31 each of naked and hulled barley from the Korea Seed and Variety Service, and 20 of malting barley from the RDA-Genebank Information Center. A cDNA library was constructed from a representative cultivar, Gwanbori, to facilitate analysis of genetic relationships, and 58 EST-SSR markers were developed and characterized. In total, 47 SSR markers were employed to analyze polymorphisms. A relationship dendrogram based on the polymorphism data was

Fig. 2 Dendrogram derived from 62 EST-SSR markers for 82 barley varieties. The red, green, and blue cultivars are the kinds of naked, hulled, and malting barley. The blue line classifies barley cultivars with 80% similarity within barley cultivars

constructed to compare genetic diversity. We determined that the PIC among cultivars is 0.519, which indicates that there is low genetic diversity among Korean barley cultivars.

Acknowledgements This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through the Agri-Bio Industry Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (313043-03-3-HD050).

Compliance with ethical standards

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

References

Aggarwal RK, Hendre PS, Varshney RK, Bhat PR, Krishnakumar V, Singh L (2007) Identification, characterization and utilization of

- EST-derived genic microsatellite markers for genome analyses of coffee and related species. *Theor Appl Genet* 114:359–372
- Bernet GP, Bramardi S, Calvache O, Carbonell EA, Asins MJ (2003) Applicability of molecular markers in the context of protection of new varieties of cucumber. *Plant Breed* 122:146–152
- Bungartz A, Klaus M, Mathew B, Léon J, Naz AA (2016) Development of new SNP derived cleaved amplified polymorphic sequence marker set and its successful utilization in the genetic analysis of seed color variation in barley. *Genomics* 107:100–107
- Fiust A, Rapacz M, Wójcik-Jagła M, Tyrka M (2015) Development of DArT-based PCR markers for selecting drought-tolerant spring barley. *J Appl Genet* 56:299–309
- Gailing O, Bodénès C, Finkeldey R, Kremer A, Plomion C (2013) Genetic mapping of EST-derived simple sequence repeats (EST-SSRs) to identify QTL for leaf morphological characters in a *Quercus robur* full-sib family. *Tree Genet Genomes* 9:1361–1367
- Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. *Theor Appl Genet* 83:250–256
- Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113:163–185
- Jia Q, Zhu J, Wang J, Yang J, Zhang G (2016) Genetic mapping and molecular marker development for the gene Pre2 controlling purple grains in barley. *Euphytica* 208:215–223
- Karsai I, Szűcs P, Mészáros K, Puskás K, Bedő Z, Veisz O (2007) Barley (*Hordeum vulgare* L.) marker linkage map: a case study of various marker types and of mapping population structure. *Cereal Res Commun* 35:1551–1562
- Kleinohs A, Kilian A, Maroof MS, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. *Theor Appl Genet* 86:705–712
- Kwon YS, Hong JH, Choi KJ (2011) Genetic diversity of Korean barley (*Hordeum vulgare* L.) varieties using microsatellite markers. *Korean J Breed Sci* 43:243–250
- Li JZ, Sjakste TG, Röder MS, Ganai MW (2003) Development and genetic mapping of 127 new microsatellite markers in barley. *Theor Appl Genet* 107:1021–1027
- Macaulay M, Ramsay L, Powell W, Waugh R (2001) A representative, highly informative 'genotyping set' of barley SSRs. *Theor Appl Genet* 102:801–809
- Miah G, Rafii MY, Ismail MR, Puteh AB, Rahim HA, Islam KhN, Latif MA (2013) A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *Int J Mol Sci* 14:22499–22528
- Morgante M, Hanafey M, Powell W (2002) Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nat Genet* 30:194–200
- Nandha PS, Singh J (2014) Comparative assessment of genetic diversity between wild and cultivated barley using gSSR and EST-SSR markers. *Plant Breed* 133:28–35
- Pedersen C, Linde-Laursen I (1995) The relationship between physical and genetic distances at the Hor1 and Hor2 loci of barley estimated by two-colour fluorescent in situ hybridization. *Theor Appl Genet* 91:941–946
- Pillen K, Binder A, Kreuzkam B, Ramsay L, Waugh R, Förster J, Léon J (2000) Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars. *Theor Appl Genet* 101:652–660
- Ponyared P, Ponsawat J, Tongsimma S, Seresantakul P, Akkasaeng C, Tantisuwichwong N (2016) ESAP plus: a web-based server for EST-SSR marker development. *BMC Genomics* 17(Suppl 13):1035
- Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. *Trends Plant Sci* 96:1360–1385
- Ramsay L, Macaulay M, Degli Ivanisovich S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuveson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganai M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. *Genet* 156:1997–2005
- Ramsay L, Russell J, Macaulay M, Booth A, Thomas WTB, Waugh R (2004) Variation shown by molecular markers in barley: genomic and genetic constraints. *Aspects Appl Biol* 72:147–154
- Rohlf FJ (2000) NTSYSpc. Numerical Taxonomy and Multivariate Analysis System-Version 2.10b. Applied Biostatistics Inc., New York
- Rudd S (2003) Expressed sequence tags: alternative or complement to whole genome sequences? *Trends Plant Sci* 8:321–329
- Shan X, Blake TK, Talbert LE (1999) Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. *Theor Appl Genet* 98:1072–1078
- Shariflou MR, Hassani ME, Sharp PJ (2001) A PCR-based DNA marker for detection of mutant and normal alleles of the Wx-D1 gene of wheat. *Plant Breed* 120:121–124
- Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Michell SE, Kresovick S, Ziegler J (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparison with data from RFLPs and pedigree. *Theor Appl Genet* 95:163–173
- Sneath PHA, Sokal RR (1973) Numerical taxonomy: the principles and practice of numerical classification. Freeman WH, San Francisco
- So EH, Choi SJ, Ko EB, Song IH, Lee JH (2004) Hordein fingerprinting for cultivar discrimination in national list of barley. *Korean J Crop Sci* 49:256–260
- Thiel T, Michalek W, Varshney R, Graner A (2003) Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theor Appl Genet* 106:411–422
- Varshney RK, Marcel TC, Ramsay L, Russell J, Röder MS, Stein N, Waugh R, Langridge P, Niks RE, Graner A (2007) A high density barley microsatellite consensus map with 775 SSR loci. *Theor Appl Genet* 114:1091–1103
- Wright JM, Bentzen P (1995) Microsatellites: genetic markers for the future. *Molecular genetics in fisheries*. Springer, Netherlands, pp 117–121
- Zhang M, Mao W, Zhang G, Wu F (2014) Development and characterization of polymorphic EST-SSR and genomic SSR markers for Tibetan annual wild barley. *PLoS One* 9(4):e94881
- Zhou G, Zhang Q, Tan C, Zhang X, Li C (2015) Development of genome-wide InDel markers and their integration with SSR, DArT and SNP markers in single barley map. *BMC Genomics* 16:804