TRANSFERABILITY AND POLYMORPHISM OF BARLEY MICROSATELLITE MARKERS ACROSS H-GENOME CONTAINING SPECIES IN THE GENUS HORDEUM (H. VULGARE AND H. BULBOSUM)

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Limited numbers of microsatellite markers are available for genetic characterization of *Hordeum bulbosum* which comprises the secondary genepool of cultivated barely. The objective of this study was to evaluate the transferability of microsatellite markers from *H. vulgare* to *H. bulbosum* and a preliminary evaluation of their polymorphism. From ninety-three pairs barley SSR primer tested for transferability, all of them amplified DNA segments in *H. vulgare* (11 accessions) and 48 pairs (51.61%) were transferable to the *H. bulbosum* (5 accessions) with high level of polymorphism. Twenty-two (23.65%) SSR markers showed transferability to *H. murinum* used as outgroup. A total of 546 alleles were detected by 48 transferred primer pairs in all accessions. The number of alleles per locus ranged from 3 to 13 with an average of 11.375 alleles per locus. The PIC values were ranged from 0.161 to 0.621 with an average of 0.477. The value of PIC in *H. vulgare* (average PIC = 0.639) was significantly higher than *H. bulbosum* (average PIC = 0.316). In dendrogram generated based on SSR data accessions were divided into groups related to their taxonomic classifications, indicating the efficiency of barley SSRs for phylogenetic analyses in H genome containing species in the genus *Hordeum*. Based on the results of this study, it can be suggested that the cross species transferable barley SSRs are valuable molecular tools, for genetic diversity analyses in the *H. bulbosum* for which limited number of microsatellite markers are available. This study provided a set of efficient SSR markers from publicly available barley microsatellite markers for the genetic characterization of *H. bulbosum*.

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قابلیت انتقال و تنوع ریزماهوارک‌های جو زراعی به گونه‌های واجد ژنوم H در جنس جو (Hordeum vulgare, H. bulbosum)

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تعداد محدودی مارکرهای ریزماهوارک مناسب برای بررسی تنوع وراثتی گونه‌ی *H. bulbosum* وجود دارد. در این مطالعه قابلیت انتقال ریزماهوارک‌های جو زراعی به گونه‌ی *H. bulbosum* و تنوع پذیری آنها مورد بررسی قرار گرفته است. از ۹۳ زوج پرایمر SSR آزمایش شده همگی در گونه‌ی *H. vulgare* (۱۱ نمونه بذر) قطعاتی از DNA را تکثیر نمودند ولی تنها ٤٦ زوج از آنها (۵۱/٦۱٪) قابلیت انتقال به گونه *H. bulbosum* (۵ نمونه بذر) با سطح قابل ملاحظه‌ای از تنوع را نشان دادند. در گونه‌ی *H. murinum* که به عنوان برون گروه استفاده شده بود ۲۲ زوج پرایمر SSR (۲۳/٦۵٪) قابلیت انتقال نشان دادند. در PCR انجام شده با استفاده از ٤۸ مارکر SSR تعداد ۵٤٦ آلل در کل جمعیت‌ها تشخیص داده شد. تعداد آلل‌ها در هر جایگاه وراثتی SSR بین ۳ تا ۱۳ با میانگین ۱۱/۳۷۵ آلل بود. مقدار PIC محاسبه شده برای هر مارکر بین ۰/۱٦۱ تا ۰/٦۲۱ با میانگین ٤۷۷/. بود. مقدار PIC در گونه‌ی *H. vulgare* (با میانگین ۰/٦۳۹) به طور معنی داری

بالاتر از *H. bulbosum* (با میانگین ۰/۳۱۶) بود. در دندروگرام حاصل جمعیت‌ها بر اساس گروه های تاکسونومیک از هم جدا شدند که نشان‌دهنده‌ی کارآیی این مارکرها برای مطالعه فیلوژنی درون این گروه است. نتایج این مطالعه نشان می‌دهد که مایکروساتلایت‌های جو زراعی برای بررسی تنوع وراثتی گونه‌ی *H. bulbosum* مناسب هستند.

Introduction

The genus *Hordeum* is classified into 32 species and about 51 cytotypes exist at three ploidy levels (2x, 4x and 6x) with a basic chromosome number of x = 7 (Bothmer et al. 1995). Genomic differentiation followed by interspecific hybridizations and polyploidizations resulted in a range of genomes and genomic constitutions within this genus. Based on the genomic constitution, the genus is classified into five genomic groups, namely H, I, X, Y and XI (Taketa et al. 1999). In this study, genome designation follows that of Taketa et al. (2001), namely, *H. vulgare* L. and *H. bulbosum* L. both carry the H genome, *H. marinum* Huds. carries the X genome, *H. murinum* L. has the Y genome, and the 25 remaining species share variants of the I genome (Taketa et al. 2005). The H genome containing species comprise the primary and secondary genepool of cultivated barley; therefore, they are of highest value in the genus. Cultivated barley (*H. vulgare* subsp. *vulgare*) and its wild progenitor (*H. vulgare* subsp. *spontaneum* C. Koch.), that considered as the primary genepool of barley; belong to a single annual diploid species (Asfaw and Bothmer 1990). Other H genome containing species, *H. balbosum,* is a perennial and obligatory outbreeding with a self incompatibility system, di-and tetraploid species that comprise secondary genepool of cultivated barely (Bothmer et al. 1995).

The potential value of *H. bulbosum* as a genetic resource for barley breeding was indicated in many reports (Pickering 1992). It has been reported that *H. bulbosum* harbors useful resistance genes such as resistance to powdery mildew (Kasha et al. 1996), leaf rust (Pickering et al. 2000) and the soilborne virus complex (Ruge et al. 2000), which can be incorporated to barley improvement.

Evaluation of variation within this genepool is fundamental for designing a strategy for its germplasm collection and conservation, identifying populations of highest conservation priority and for tracking the origin of domesticated barley. Morphological characters are not precise indicators of genetic potential of a germplasm, therefore, using molecular markers we may reveal hidden genetic diversities.

Among several molecular marker systems developed so far, microsatellites have become the marker of choice in many recent investigations due to their high reproducibility and polymorphism. Designing microsatellite markers is a critical time and fund consuming step and therefore the specific SSR markers for many of the species are not available. A parsimonious crosscut way is choosing microsatellites through testing available microsatellite markers as they are transferable to close congener species and have limited transferability to species of other genera (Ellis and Burke 2007).

The successful transferability of microsatellite primers from *Theobroma cacao* to *Theobroma grandiflorum* (Alves et al. 2006), from cultivated peanut (*Arachis hypogaea*) to the other congener species (Bravo et al. 2006; Gimenes et al. 2007), from *Hordeum vulgare* to *H. chilense* Brongn. (Castillo et al. 2008), from *Triticum astivum* L. to *Triticum dicoccoides* (Koern. ex Ascherson & graebner) Aaronsohn (Fahima et al. 1998), from *Secale cereale* L. to *S. strictum* (Jenabi et al. 2011) and from *Festuca arundinacea* Scherb. to *Lolium persicum* Boiss. & Hohen. ex Boiss. (Sharifi Tehrani et al. 2008) were indicated with different level of polymorphism and phylogenetic inference.

The phylogenetic relationships of *H. vulgare* and *H. bulbosum* have not been studied in detail so far using SSRs. Regarding the importance of *H. bulbosum* as a gene sources and lack of available SSR markers for evaluating its genetic diversity, this study was aimed to estimate transferability and polymorphism of barley SSRs across H genome containing species, *H. vulgare* and *H. bulbosum*, and their potential use as molecular tools for introgression and variability analysis.

Material and Methods

A total of 17 accessions of H genome containing species of the genus *Hordeum*: 5 accessions of *H. bulbosum* (HB), 3 accessions of *H. vulgare* subsp. *vulgare* var. *distichon* (l.) Alef (HD), 4 accessions of *H. vulgare* subsp. *vulgare* var. *hexastichon* (L.) Aschers. (HH), 4 accessions of *H. vulgare* subsp. *spontaneum* (HS) and one accession of *H. murinum* subsp. *glaucum* (Steud.) Tzvel. (HM) used as outgroup (Table 1) were and geographic origin of accessions used in this study.

Table 1. Taxon, ploidy level, accession codes, altitude (m) W; west, SW; southwest, N; north, NE; northeast. HS (*H. vulgare* subsp. *spontaneu*m), HD (*H. vulgare* subsp. *vulgare* var. *distichon*), HH (*H. vulgare* subsp. *vulgare* var. *hexastichon*), HB (*H. bulbosum*).

Taxon	Ploidy level	Accession code	Region & Province	Locality & Altitude
H.vulgare subsp. *vulgare* var. *hexastichon*	2n=2x=14	HHcham	W: Lorestan	Poledokhtar, Chamemehr, 852 m
		HHabsh	SW: Esfahan	Semirom, Abshar, 2362 m
		HHnek	N: Mazanderan	Sari toward Neka,5km, 43 m
		HHbadr	NE: Khorasan-e shomali	Ashkhaneh toward Bojnoord, Badranloo, 915 m
H.vulgare subsp. *vulgare* var. *distichon*	2n=2x=14	HDdom	W: Lorestan	Khoramabad toward Poledokhtar, Domrud, 907 m
		HDarj	SW: Fars	Shiaz toward kazerun, Dashte Arjan, 2051 m
		HDsisb	NE: Khorasan-e shomali	Bojnurd, Sisab toward Nodeh, 1288 m
H.vulgare subsp. *spontaneum*	2n=2x=14	HSdar	W: Ilam	Darehshahr, Shahr-e bastani, 690 m
		HSteh	N: Tehran	Boomehen, 1640 m
		HSgol	NE: Golestan	National Park of Golestan, 900 m
		HSbab	SW: Kohgilooie va Boyerahmad	Babameidan,the first Turn, 1746 m
H. bulbosum	2n=4x=28	HBdar	W: Ilam	Darehshahr, Shahr-e bastani, 690 m
		HBdzan	SW: Fars	Eghlid toward Marvdasht, Dorudzan, 1690 m
		HBabali	N: Tehran	Abali, 2127 m
		HBkhosh	NE: Golestan	Azadshahr toward shahrood, Khoshyeilagh, 1775 m
		HBheir	NW: Gilan	Astara, Gardanei-e Heiran, 1537 m
H. murinum subsp. *glaucum*	2n=2x=14	HMsah	W: Kermanshah	Sahneh, Sarab-e Sahneh, 1450 m

analysed. Accessions were collected from various regions of Iran and these were identified morphologically according to Bothmer et al. (1995).

From each accession 15 – 20 seeds were grown in experimental field and DNA was isolated from fresh leaves according to Komatsuda et al. (1998). Ninety three primer pairs flanking microsatellites ("primers") derived from *Hordeum vulgare* (Ramsay et al. 2000; Liu et al. 1996) were used to evaluate transferability of barley microsatellites across species. Marker names, primer sequences, chromosomal locations and other details regarding microsatellites are listed in Table 2.

PCR amplification were carried out in 10 µL, containing approximately 50 ng template genomic DNA, 250 nM of each primer (see Table 2), 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1.2 U EX-*Taq* polymerase (Takara, Tokyo, Japan). PCR programs were performed as described by Liu et al. (1996) and Ramsay et al. (2000) with minor modifications as below:

Program 1 – After initial denaturation at 94 ºC for 5 min, ten cycles were performed at 94 ºC for 1 min, at 63°C for 1 min, and at 72 ºC for 1 min, followed by 30 cycles with the lowered annealing temperature (55 °C); followed by a final extension step of 7 min at 72 ºC.

Table 2. Sequence, repeat motif, PCR programs (shown by numbers 1, 2, 3 and 4; for details see text) and allele size range (bp) of the 93 SSR loci tested for transferability in this study. NA (not amplified), Chr. (chromosome location) HV (*Hordeum vulgare*), HB (*Hordeum bulbosum*) and HM (*Hordeum murinum*).

SSR locus	Published data (Ramsay et al. 2000; Liu et al. 1996)						Allele size range (this study)		
	Forward primer (5'-3')	Reverse primer (5'-3')	Repeat motif	PCR Program	Chr.	Allele size (bp)	HV	HB	HM
Bmac0032	CCATCAAAGTCCGGCTAG	GTCGGGCCTCATACTGAC	(AC)7T(CA)15(AT)9	3	1H	215	210-300	110-250	NA
Bmac0154	CTGGGTGATGAATAGAGTTTC	TATTCTTCAAAAGATGTTCTGC	(AT)19/(AC)6	4	1H	130	130-180	120-200	NA
Bmac0213	ATGGATGCAAGACCAAAC	CTATGAGAGGTAGAGCAGCC	(AC)23	4	1H	168	140-200	150-180	250-400
Bmac0399	CGATGCTTTACTATGAGAGGT	GGGTCTGAAGCCTGAAC	(AC)21	3	1H	145	140-180	NA	NA
Bmag0211	ATTCATCGATCTTGTATTAGTCC	ACATCATGTCGATCAAAGC	(CT)16	4	1H	174	168-200	160	NA
HvHVA1	CATGGGAGGGGACAACAC	CGACCAAACACGACTAAAGGA	(ACC)5	2	1H	136	130-136	124-150	NA
HVM20	CTCCACGAATCTCTGCACAA	CACCGCCTCCTCTTTCAC	(GA)19	1	1H	151	150-165	260-300	NA
WMC1E8	TCATTCGTTGCAGATACACCAC	TCAATGCCCTTGTTTCTGACCT	(AC)24	2	1H	197	172-270	170-178	172
Bmac0093	CGTTTGGGACGTATCAAT	GGGAGTCTTGAGCCTACTG	(AC)24	2	2H	151	110-160	260-300	NA
Bmac0134	CCAACTGAGTCGATCTCG	CTTCGTTGCTTCTCTACCTT	(AC)28	2	2H	148	140-160	NA	200-700
Bmag0125	AATTAGCGAGAACAAAATCAC	AGATAACGATGCACCACC	(AG)19	2	2H	134	130-150	140-155	180-500
Bmag0378	CTTTTGTTTCCGTAGCATCTA	ATCCAACTATAGTAGCAAAGCC	(AG)14	4	2H	147	140-155	NA	NA
Bmag0381	TTTTATTATTGCATCTAGGGC	TATCAAGATCATGACGTCTCA	(CT)7(ATCT)6 (CT)14	3	2H	141	130-150	NA	NA
Bmag0518	AATGCCATGATGTTATTGG	AAGAAGATTACATCGAATAGATCA	(TC)23	4	2H	168	160-175	NA	NA
Bmag0692	GCAAGGTATCTCTTGTATTTTG	TGGCATCTACAATCTAAAACA	(CT)19	2	2H	182	170-210	188-200	NA
EBmac0415	GAAACCCATCATAGCAGC	AAACAGCAGCAAGAGGAG	(AC)17	2	2H	247	240-300	240-310	280
EBmac0521	TGAAGCACAAGTGTGGAA	AGACGTATCATTGCCCAC	(AC)18	2	2H	163	110-180	110-130	200-700
EBmac0557	ATGCATGTGTAGATGTAGATGTG	AACAAGGATAAACTAACATGGG	(AC)8	3	2H	154	147-170	110-170	157
EBmac0607	GCGAACATTGTCATGTTAGTA	AACCTTATGGATTTGGAGG	(TG)7(TG)6(GA)10 (AG)6	2	2H	146	140-175	110-210	NA
EBmag0793	ATATATCAGCTCGGTCTCTCA	AACATAGTAGAGGCGTAGGTG	(GT)13(AG)36	2	2H	177	165-185	NA	270-700
HVHOTR1	ATGAGCAGTCTTGTCTTAACC	AGTTGGTCGCTAGATCTTATG	(CAA)6	2	2H	165	165-200	190-215	NA
HvTUB	CATTCTTTACACCCAAGAAAG	CAGTAGAGCTCCCAGCAA	(AGC)8	2	2H	149	149	NA	NA
Bmac0067	AACGTACGAGCTCTTTTTCTA	ATGCCAACTGCTTGTTTAG	(AC)18	2	3H	171	110-280	110-120	NA
Bmag0006	TTAAACCCCCCCCCTCTAG	TGCAGTTACTATCGCTGATTTAGC	(AG)17	4	3H	174	165-240	165-200	250-400
Bmag0013	AAGGGGAATCAAAATGGGAG	TCGAATAGGTCTCCGAAGAAA	(CT)21	4	3H	155	150-180	NA	NA
Bmag0131	TTTCAGAAACGGAGTTTTG	CCTCCACACAAAAAATCC	(AG)16G(AG)15	2	3H	149	140-180	NA	NA
Bmag0508A	TCTCCGTATATTTAGGAAACG	TATCTCCCCCTAGATAGAAGG	(AG)14	2	3H	175	170-180	160-170	170
Bmag0603	ATACCATGATACATCACATCG	GGGGGTATGTACGACTAACTA	(AG)24	2	3H	120	110-145	NA	NA
Bmag0606	CTATTTGTAATGTATGTATGTCCC	TCATTGGTCCAGATAATACAA	(CT)22	2	3H	140	130-170	NA	NA
EBmac0871	TGCCTCTGTTGTGTTATTGT	CCCCAAGTGAACATTGAC	(TG)13	2	3H	180	170-195	NA	NA
EBmag0705	TCCTGTAGTCCTCTTTGTTTC	TATATTACACATGGAGAGAGAGG	(TC)31	2	3H	155	140-170	NA	NA
HvLTPPB	AGACGCTGAGTACGTTGAG	CAAAGTACAACAAACTCACGA	(AC)10(AT)5	2	3H	221	190-280	190-280	NA
HVM70	CCGCCGATGACCTTCTC	ACCCACGACCTATGGCAC	(CA)8	2	3H	154	145-165	NA	NA
Bmac0181	ATAGATCACCAAGTGAACCAC	GGTTATCACTGAGGCAAATAC	(AC)20	2	4H	177	165-185	NA	NA
Bmac0310	CTACCTCTGAGATATCATGCC	ATCTAGTGTGTGTTGCTTCCT	(CT)11(AC)20	2	4H	176	160-190	NA	NA
Bmag0014	GCAGGGGTTGAACATCTCAT	CACAGGGAAACAGCTATGACC	(CT)15	2	4H	142	140-150	NA	NA
Bmag0375	CCCTAGCCTTCCTTGAAG	TTACTCAGCAATGGCACTAG	(AG)19	4	4H	135	120-140	NA	NA
Bmag0384	TGTGAGTAGTTCACCATAGACC	TGCCATTATCATTGTATTGAA	(AG)18	4	4H	116	105-116	105-116	180
Bmag0490	TGATACATCAAGATCGTGACA	GGGACTGAGTGTATGAATGAG	(AG)24	2	4H	121	110-140	100-130	NA
EBmac0679	ATTGGAGCGGATTAGGAT	CCCTATGTCATGTAGGAGATG	(AC)22	2	4H	148	140-175	140-160	NA
EBmac0701	ATGATGAGAACTCTTCACCC	TGGCACTAAAGCAAAAGAC	(AC)23	2	4H	149	130-160	150-170	NA
EBmac0775	GCTTCCTTCATAGACCCAT	ATATCATGCCAATGGTGTC	(TG)4TT(TG)17 (AG)10	2	4H	149	140-170	100-130	100-150
EBmac0788	TAACTTACTTTATATCCATGGCA	ATGATGAGAACTCTTCACCC	(TG)23	3	4H	168	160-185	NA	NA
EBmac0906	CAAATCAATCAAGAGGCC	TTTGAAGTGAGACATTTCCA	(GC)5GGG(GT)16	3	4H	153	150-180	NA	NA
EBmag0781	CTATTTTCTAATGCTTGGACC	TGTCTAGTTCATCATCATTGC	(CT)21	2	4H	149	145-195	NA	NA
HVMLOH1A	CCTCCCCTCTGATATGATAA	GTACAGACGGTTTAATTGTCC	(GA)6	2	4H	175	140-180	185-220	NA
HVM03	ACACCTTCCCAGGACAATCCATTG	AGCACGCAGAGCACCGAAAAAGTC	(AT)29	2	4H	188	160-260	166-195	150-210
HVM40	CGATTCCCCTTTTCCCAC	ATTCTCCGCCGTCCACTC	(GA)6(GT)4(GA)7	1	4H	160	150-170	300-350	400-500
HVM67	GTCGGGCTCCATTGCTCT	CCGGTACCCAGTGACGAC	(GA)11	1	4H	116	116-260	NA	NA
AF043094A	CACGGTATAAATATCCACCC	ATGGACTCTTCTCCCTGAA	(CTGT)5	4	5H	146	145-160	NA	NA

Table 2. Continued. Sequence, repeat motif, PCR programs (shown by numbers 1, 2, 3 and 4; for details see text) and allele size range (bp) of the 93 SSR loci tested for transferability in this study. NA (not amplified), Chr. (chromosome location) HV (*Hordeum vulgare*), HB (*Hordeum bulbosum*) and HM (*Hordeum murinum*).

SSR locus	Published data (Ramsay et al. 2000; Liu et al. 1996)						Allele size range (this study)		
	Forward primer (5'-3')	Reverse primer (5'-3')	Repeat motif	PCR Program	Chr.	Allele size (bp)	HV	HB	HM
Bmac0096	GCTATGGCGTACTATGTATGGTTG	TCACGATGAGGTATGATCAAAGA	(AT)6(AC)16	4	5H	173	170-200	NA	NA
Bmac0163	TTTCCAACAGAGGGTATTTACG	GCAAAGCCCATGATACATACA	(AC)6(GC)3(AC)17	2	5H	146	140-170	NA	NA
Bmag0222	ATGCTACTCTGGAGTGGAGTA	GACCTTCAACTTTGCCTTATA	(AC)9(AG)17	4	5H	179	170-195	NA	NA
Bmag0323	TTTGTGACATCTCAAGAACAC	TGACAAACAAATAATCACAGG	(CT)24	2	5H	158	150-190	NA	NA
Bmag0337	ACAAAGAGGGAGTAGTACGC	GACCCATGATATATGAAGATCA	(AG)22	2	5H	145	140-170	NA	NA
EBmac0518	ATATGGGTCACACTGAAAATC	AGTTTGTTTTTACCAATAAGAGTG	(AC)5/(AC)5	2	5H	150	150-165	150	NA
EBmac0684	TTCCGTTGAGCTTTCATACAC	ATTGAATCCCAACAGACACAA	(TA)7(TG)11(TG)11 (TTTG)5	2	5H	172	170-200	280	NA
EBmac0970	ACATGTGATACCAAGGCAC	TGCATAGATGATGTGCTTG	(AC)8	2	5H	112	110-120	200-250	NA
EBmatc0040	AAAGTTGACCACCACTGTTGA	ATGATGATGGTCTTTCTTCTGG	(ATC)6N3(ATC)3	3	5H	179	175-195	180-205	185
EBmatc0054	TGACCACCATTGTGAGACAG	AGTGGTAGTGGGAGGAGGAG	(GGA)3(ATC)4	3	5H	128	124-200	124-210	125
HvLOX	CAGCATATCCATCTGATCTG	CACCCTTATTTATTGCCTTAA	(AG)9	4	5H	150	145-155	NA	NA
Bmac0018	GTCCTTTACGCATGAACCGT	ACATACGCCAGACTCGTGTG	(AC)11	3	6H	138	135-155	NA	NA
Bmac0316	ATGGTAGAGGTCCCAACTG	ATCACTGCTGTGCCTAGC	(AC)19	2	6H	135	130-170	NA	NA
Bmag0009	AAGTGAAGCAAGCAAACAAACA	ATCCTTCCATATTTTGATTAGGCA	(AG)13	4	6H	172	165-185	NA	NA
Bmag0173	CATTTTTGTTGGTGACGG	ATAATGGCGGGAGAGACA	(CT)29	4	6H	150	150-250	110-140	NA
Bmag0496	AGTATAACCAACAGCCGTCTA	CTATAGCACGCCTTTGAGA	(CT)20	2	6H	189	180-210	NA	NA
Bmag0500	GGGAACTTGCTAATGAAGAG	AATGTAAGGGAGTGTCCATAG	(AG)6CG(AG)29 (AGAGGG)3(AG)6	4	6H	150	140-170	NA	200-500
Bmag0613	AAGAACACCATATGATCCAAC	CTCCATGACTATGAGGAGAAG	(GA)17	2	6H	171	154-220	154-192	160
EBmac0602	GATTGGAGCTTCGGATCAC	CCGTCTAGGGAGAGGTTCTC	(AC)9AT(AC)7 (AG)9	4	6H	205	170-248	175-205	190
EBmac0674	GAACGTATAGCAGGAGCAA	CATCGTTCCCTTCATGAT	(TG)18(AG)9	2	6H	147	146-160	150-165	NA
EBmac0806	ACTAAGTCCTTTCACGAGGA	GTGTGTAGTAGGTGGGTACTTG	(CA)4GA(CA)8 (CA)5	2	6H	168	160-180	160-190	NA
AF022725A	AGTATGGGGAATTTATTTGG	GCTGCAAAGTATGACAATATG	(TG)8	2	7H	136	130-160	135-170	NA
Bmac0031	AGAGAAAGAGAAATGTCACCA	ATACATCCATGTGAGGGC	(AC)28	3	7H	175	175-215	150-195	150
Bmac0167	CATTTCCACTTCAAAATATCC	CCAAAGTTTGAGTGCAGAC	AC)20	2	7H	184	180-190	NA	NA
Bmac0224	GCATATATACCACCCTTGGT	ATTCTTGATGGCTATAGCTTG	(AC)5/(AC)5	2	7H	166	166	NA	NA
Bmac0273	ACAAAGCTCGTGGTACGT	AGGGAGTATTTCACCCTTG	(AC)20(AG)20	2	7H	186	175-190	170	NA
Bmag0007	TGAAGGAAGAATAAACAACCAACA	TCCCCTATTATAGTGACGGTGTG	(AG)16(AC)16	4	7H	185	180-200	NA	NA
Bmag0011	ACAAAAACACCGCAAAGAAGA	GCTAGTACCTAGATGACCCCC	(AG)25	4	7H	147	140-210	200-240	NA
Bmag0021	ATTTTTATCAGAACGTCTCTCTC	CTAACTTCTCTCTCCCTCTCC	(CA)10AA(GA)28	4	7H	143	130-170	NA	NA
Bmag0120	ATTTCATCCCAAAGGAGAC	GTCACATAGACAGTTGTCTTCC	(AG)15	4	7H	230	225-250	NA	270-300
Bmag0135	ACGAAAGAGTTACAACGGATA	GTTTACCACAGATCTACAGGTG	(AG)10GG(AG)12	4	7H	161	115-224	124-210	124
Bmag0189	GAATGAAAAACACGAGGTAAC	AGATTGAACTCAACTCAAGGA	(CT)21	2	7H	151	140-160	NA	NA
Bmag0206	TTTTCCCCTATTATAGTGACG	TAGAACTGGGTATTTCCTTGA	(GT)5(AG)14	4	7H	239	235-265	NA	NA
Bmag0217	AATGCTCAAATATCTATCATGAA	GGGGCTGTCACAAGTATATAG	(AG)19	4	7H	196	180-200	NA	NA
Bmag0341	TCATGGAGACCGTTGTAGT	CCACAAGCCTCTGTTCTC	(AG)14	2	7H	214-228	230-270	230-270	NA
Bmag0369	CACTAGGCACCAATGACTG	ATCGAAAATCTTAGCTTTGG	(CT)16	4	7H	191	185-200	NA	NA
Bmag0516	ATCTAACCCGAACCTTGAG	AGCATCCATATATACAATGATACA	(TC)8(TATC)7 (TC)19	4	7H	147	140-150	NA	NA
EBmac0755	AGCCTTGTGTATCAGGACA	CTGCTGGTGTTCTCTAAAAGT	(AC)16	2	7H	143	130-165	130-165	NA
EBmac0827	CATGGTATTCAAACATACACG	AAGGTCTTAAGGGGTGATG	(CA)15TG(TA)7	2	7H	112	120-150	110-140	NA
EBmag0794	CAGTCATAACCTGATGAACAA	TCACACTTATCTTGCTGCTAA	(TA)23(GA)16	2	7H	197	150-210	127-140	NA
EBmatc0016	CCAACCAAGATAATGTGCTTG	ATCCTTATGCTCCTCGCTG	(ATC)4N9(ATC)12	3	7H	143	140-160	140-170	NA
HVM04	AGAGCAACTACCAGTCCAATGGCA	GTCGAAGGAGAAGCGGCCCTGGTA	(AT)9	2	7H	198	190-230	NA	NA
HVPLASC1B	GTGCATGCATCATATTGATTA	ACGTACGTACTTATCACGAAGA	(TCTAC)4	2	7H	110	110-150	100-120	NA

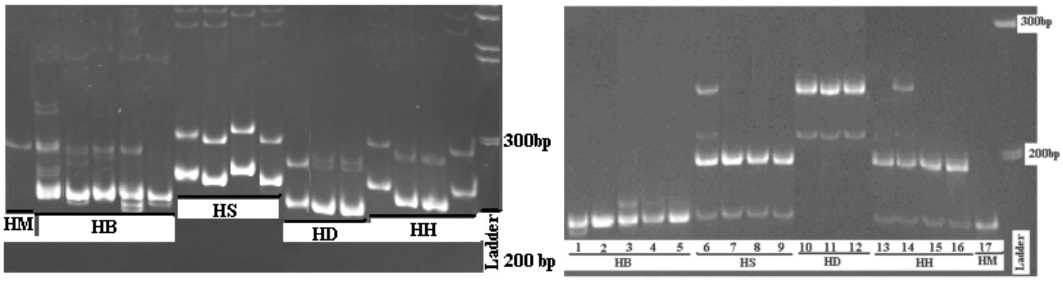

Fig. 1. Representative SSR gel images depicting the reaction products from PCR amplifications of genomic DNA from 17 accessions of H genome containing *Hordeum* species (Table 2) with SSR primers (a) EBmac0415 and (b) WMC1E8. The pattern of allelic diversity is clearly correlated with the recognized taxa. HS (*H. vulgare* subsp. *spontaneu*m), HD (*H. vulgare* subsp. *vulgare* var. distichon), HH (*H. vulgare* subsp. *vulgare* var. *hexastichon*), HB (*Hordeum bulbosum*).

Program 2 – After initial denaturation at 94 ºC for 5 min , 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, and extension for 30 s at 72 °C, followed by a final extension for 7 min at 72 °C.
Program 3 – Identical to program 1 except that annealing temperatures were 64 °C and 60 °C respectively.
Program 4 – Identical to Program 2 except that annealing temperature was 58 °C.
In cases where either no PCR product or weak banding was observed, PCR optimization was carried out by decreasing and/or increasing the annealing temperatures, and switching to "touchdown" PCR conditions.

Along with size marker tracks (50 bp DNA ladder, Promega), PCR products were mixed with loading buffer (2:1) and loaded on 2% agarose gel for initial information about produced amplicons (Table 2). Where the primers could amplified fragments of DNA, PCR products were separated on 12% non-denaturing polyacrylamide gels at 300 mA for 180 min in 1× TBE buffer, and visualized by ethidium bromide (0.5 mg/ml) staining and UV light (following Wang et al. 2003). Gels were scanned into Adobe Photoshop (Fig. 1) and band sizes entered into a scoring matrix.

A binary matrix was generated, where the presence or absence of each allele was coded by 1 or 0 respectively and row data were recorded in a scoring matrix generated by Microsoft Excel. Microsatellite data were analysed using PowerMarker software ver 3.25 (Nei and Takezaki 1983) and NTSYS-pc software ver. 2.1 (Rolf 2000). Polymorphism information content (PIC) which is a measure of allelic variability and evenness at a particular locus was calculated for each locus as described by Anderson et al. (1992) (PIC = $1-\sum (P_i)^2$, where P_i is the proportion of samples carrying the *i*th allele of a particular locus). Allele number per locus was also calculated. Similarities among the accessions were calculated according to Dice coefficient (Dice 1945) using SIMQUAL module in NTYSYS-PC software version 2.1 (Rolf 2000) (Fig. 2). The scores of microsatellite alleles and calculated genetic distances were used to generate UPGMA dendrogram showing relationships among taxa. Trees based on other similarity coefficients, bootstrap values and neighbor joining methods were also generated which showed no significant differences in topology.

Results

AMPLIFICATION AND POLYMORPHISM

Ninety-three barley microsatellite primer pairs were tested for their transferability across *H. vulgare* (different subspecies and varieties), *H. bulbosum*, and *H. murinum*. Forty-eight SSR primer pairs (51.61%) gave reproducible amplification products from all five accessions of *H. bulbosum*, and from them, 22 (23.65%) amplified in the *H. murinum* genome (Tables 1, 2 and 3; two representative SSR images are shown in Fig. 1). From these microsatellites, all of the 48 primers were polymorphic in *H. vulgare* while four primer pairs (Bmag0211, EBmac0684, Bmac0273 and EBmac0518 loci) had not polymorphism in *H. bulbosum* with only one allele per locus (Table 3). One primer pair (EBmac0602) was successful in amplifying products from only some of the accessions of *H. bulbosum* used in this study. A total of 546 alleles were detected by 48 primer pairs in all accessions studied. The number of alleles per locus ranged from three (for loci Bmag0508A and EBmac0518) to thirty alleles (for locus Bmac0032), with a mean of 11.375 alleles per locus. The PIC value was ranged from 0.161 for the HVHOTR1 locus to 0.621 for the EBmac0679 locus with an average of 0.477. From the 546 alleles detected, 380 alleles were found exclusively within the HV accessions, 241 exclusively within the HB

Table 3. Allele number, PIC (plymorphism information content) and gene diversity of the 48 SSR markers showed transferability in this study. HV (*Hordeum vulgare*), HS (*H. vulgare* subsp. *spontaneum*), HD (*H. vulgare* subsp. *vulgare* var. *distichon*), HH (*H. vulgare* subsp. *vulgare* var. *hexastichon*), HB (*Hordeum bulbosum*). Chromosomal locations, sequences, repeat motifs, PCR programs and allele size range were presented in Table 2.

	Allele size range (bp)			Allele number						PIC						Gene diversity					
SSR locus	HS	HD	HH	HS	HD	HH	HV	HB	Total	HS	HD	HH	HV	HB	Total	HS	HD	HH	HV	HB	Total
Bmac0032	210-300	230-300	250-300	10	9	8	20	12	30	0.74	0.62	0.79	0.93	0.36	0.65	0.74	0.69	0.81	0.95	0.37	0.66
Bmac0154	130-170	140-170	150-180	5	4	3	11	8	15	0.54	0.48	0.44	0.55	0.33	0.44	0.55	0.46	0.51	0.55	0.33	0.44
Bmac0213	150-195	140-200	140-195	5	4	3	10	4	11	0.68	0.64	0.61	0.88	0.36	0.62	0.81	0.70	0.71	0.89	0.37	0.63
Bmag0211	168-200	168-200	168-200	4	4	4	8	1	9	0.67	0.64	0.61	0.85	0.0	0.42	0.72	0.70	0.70	0.86	0.0	0.43
HvHVA1	130-136	130-136	130-136	2	2	2	2	4	6	0.48	0.24	0.44	0.70	0.31	0.50	0.27	0.64	0.61	0.71	0.31	0.51
HVM20	150-160	155-165	150-165	3	2	3	5	4	9	0.54	0.35	0.44	0.53	0.0	0.26	0.51	0.44	0.47	0.53	0.0	0.27
WMC1E8	172-270	220-270	172-270	4	2	4	4	4	8	0.30	0.35	0.38	0.67	0.35	0.51	0.37	0.41	0.5	0.68	0.35	0.52
Bmac0093	110-160	120-160	120-160	3	2	2	4	4	8	0.70	0.44	0.55	0.65	0.36	0.51	0.63	0.61	0.58	0.66	0.37	0.52
Bmag0125	130-150	120-150	130-145	3	3	3	6	2	6	0.74	0.70	0.63	0.86	0.34	0.60	0.71	0.71	0.69	0.84	0.35	0.6
Bmag0692	175-210	170-200	175-200	4	5	5	6	4	10	0.58	0.55	0.55	0.74	0.37	0.56	0.61	0.71	0.59	0.75	0.38	0.57
EBmac0415	260-300	240-275	240-295	6	4	4	10	12	21	0.55	0.54	0.63	0.8	0.36	0.58	0.62	0.64	0.69	0.8	0.37	0.59
EBmac0521	110-170	110-180	110-180	4	5	6	13	2	13	0.61	0.58	0.54	0.57	0.33	0.45	0.59	0.61	0.58	0.58	0.34	0.46
EBmac0557	147-170	152-170	152-165	4	4	3	8	3	11	0.44	0.34	0.31	0.38	0.52	0.45	0.34	0.38	0.37	0.39	0.53	0.46
EBmac0607	140-170	150-175	145-165	3	2	4	8	12	18	0.37	0.24	0.63	0.66	0.58	0.62	0.49	0.61	0.69	0.68	0.58	0.63
HVHOTR1	165-200	180-200	180-200	3	2	2	3	2	4	0.21	0.19	0.0	0.19	0.0	0.10	0.19	0.21	0.50	0.20	0.0	0.10
Bmac0067	110-280	140-280	140-280	6	5	5	13	3	14	0.67	0.55	0.44	0.62	0.44	0.53	0.61	0.61	0.55	0.62	0.45	0.54
HvLTPPB	190-280	195-255	220-240	5	4	3	10	12	14	0.55	0.67	0.58	0.79	0.31	0.55	0.62	0.71	0.66	0.81	0.32	0.56
Bmag0006	165-240	175-235	170-225	4	3	3	8	8	13	0.55	0.59	0.55	0.78	0.60	0.69	0.62	0.71	0.63	0.79	0.62	0.71
Bmag0508A	170-180	170	170-180	2	1	2	2	2	3	0.21	0.0	0.0	0.20	0.13	0.17	0.50	0.24	0.21	0.19	0.15	0.17
Bmag0384	105-116	105-116	105-116	3	2	2	3	2	4	0.44	0.21	0.19	0.38	0.22	0.30	0.58	0.34	0.26	0.38	0.24	0.31
Bmag0490	110-135	110-140	110-140	4	4	5	7	8	11	0.71	0.63	0.59	0.82	0.38	0.60	0.71	0.71	0.67	0.82	0.39	0.61
EBmac0679	145-165	150-165	140-175	4	2	3	5	4	9	0.74	0.71	0.63	0.83	0.41	0.62	0.74	0.71	0.68	0.84	0.42	0.63
EBmac0701	130-160	135-160	130-155	6	5	3	11	6	15	0.69	0.61	0.58	0.69	0.36	0.53	0.68	0.64	0.61	0.69	0.38	0.54
EBmac0775	140-170	150-170	150-165	5	4	3	9	4	13	0.77	0.71	0.63	0.8	0.36	0.58	0.79	0.71	0.68	0.81	0.38	0.60
HVMLOH1A	140-180	170	170	4	1	1	4	6	9	0.37	0.0	0.0	0.41	0.38	0.40	0.40	0.40	0.28	0.42	0.39	0.41
HVM03	160-260	190-250	165-220	9	6	5	19	4	20	0.7	0.62	0.75	0.94	0.30	0.62	0.78	0.71	0.78	0.96	0.31	0.63
HVM40	150-170	155-170	150-170	4	3	4	6	4	10	0.55	0.44	0.41	0.61	0.36	0.49	0.58	0.57	0.48	0.61	0.38	0.50
EBmac0518	150-165	155-165	155-165	3	2	2	3	1	3	0.55	0.44	0.38	0.49	0.0	0.24	0.47	0.44	0.41	0.48	0.0	0.24
EBmac0684	170-200	175-200	175-190	5	4	3	8	1	9	0.63	0.61	0.59	0.66	0.0	0.33	0.62	0.61	0.62	0.66	0.0	0.33

Table 3. Continued. Allele number, PIC (plymorphism information content) and gene diversity of the 48 SSR markers showed transferability in this study. HV (*Hordeum vulgare*), HS (*H. vulgare* subsp. *spontaneum*), HD (*H. vulgare* subsp. *vulgare* var. *distichon*), HH (*H. vulgare* subsp. *vulgare* var. *hexastichon*), HB (*Hordeum bulbosum*). Chromosomal locations, sequences, repeat motifs, PCR programs and allele size range were presented in Table 2.

	Allele size range (bp)			Allele number						PIC						Gene diversity					
SSR locus	HS	HD	HH	HS	HD	HH	HV	HB	Total	HS	HD	HH	HV	HB	Total	HS	HD	HH	HV	HB	Total
EBmac0970	110-120	110-120	110-120	3	3	2	3	4	8	0.59	0.44	0.37	0.53	0.34	0.43	0.51	0.49	0.39	0.53	0.35	0.44
EBmatc0040	175-195	180-195	180-195	6	3	3	6	8	12	0.55	0.36	0.31	0.54	0.43	0.48	0.59	0.51	0.44	0.54	0.43	0.49
EBmatc0054	124-200	124-190	124-190	3	2	2	4	4	7	0.33	0.34	0.0	0.43	0.41	0.42	0.35	0.40	0.34	0.43	0.41	0.42
Bmag0173	150-250	150-250	150-250	9	8	6	16	8	22	0.63	0.59	0.55	0.72	0.31	0.51	0.67	0.70	0.61	0.72	0.31	0.52
Bmag0613	154-210	160-215	165-220	7	4	4	14	4	18	0.55	0.54	0.70	0.84	0.36	0.60	0.62	0.74	0.75	0.85	0.38	0.62
EBmac0806	160-180	160-180	160-180	4	4	3	6	4	8	0.77	0.59	0.44	0.81	0.36	0.59	0.74	0.71	0.61	0.82	0.38	0.60
EBmac0602	170-248	170-240	210-230	6	5	4	10	4	10	0.79	0.54	0.22	0.81	0.33	0.57	0.81	0.70	0.48	0.82	0.34	0.58
EBmac0674	146-160	146-160	146-160	3	5	4	6	2	6	0.19	0.21	0.19	0.18	0.27	0.22	0.55	0.22	0.55	0.19	0.28	0.24
AF022725A	130-160	140-160	140-160	6	4	4	8	4	10	0.44	0.31	0.51	0.51	0.33	0.42	0.48	0.50	0.54	0.52	0.35	0.43
Bmac0031	175-210	180-215	185-210	6	5	4	10	16	24	0.55	0.45	0.47	0.67	0.51	0.59	0.62	0.61	0.53	0.67	0.52	0.60
Bmac0273	175-190	175-190	175-190	7	5	5	9	1	10	0.59	0.44	0.47	0.62	0.0	0.31	0.61	0.59	0.5	0.63	0.0	0.31
Bmag0011	140-210	150-210	150-210	4	3	3	4	4	7	0.44	0.37	0.41	0.48	0.41	0.44	0.48	0.44	0.44	0.48	0.41	0.45
Bmag0135	115-224	115-224	115-180	10	7	6	17	3	18	0.70	0.74	0.55	0.93	0.13	0.53	0.74	0.70	0.63	0.94	0.14	0.54
Bmag0341	230-270	240-270	240-260	4	4	3	6	4	10	0.71	0.66	0.73	0.84	0.30	0.57	0.75	0.71	0.76	0.84	0.32	0.58
EBmac0755	130-165	130-160	140-160	6	5	4	9	8	13	0.73	0.71	0.68	0.81	0.31	0.56	0.76	0.71	0.73	0.81	0.32	0.56
EBmac0827	120-140	120-150	120-150	5	4	4	7	8	11	0.59	0.55	0.51	0.64	0.36	0.50	0.61	0.61	0.56	0.64	0.37	0.51
EBmag0794	150-210	150-200	150-180	6	5	5	12	4	16	0.63	0.45	0.65	0.84	0.27	0.55	0.69	0.71	0.69	0.84	0.27	0.56
EBmatc0016	140-160	140-160	140-160	3	2	2	3	2	4	0.25	0.23	0.19	0.25	0.26	0.26	0.50	0.24	0.28	0.26	0.27	0.27
HVPLASC1B	100-120	100-120	110-120	3	4	3	4	4	6	0.31	0.28	0.22	0.32	0.30	0.31	0.34	0.33	0.32	0.33	0.32	0.32
Mean				4.75	3.80	3.60	7.91	4.96	11.4	0.55	0.47	0.46	0.64	0.31	0.47	0.59	0.57	0.55	0.65	0.32	0.48

accessions and 75 alleles were common in both HV and HB (Table 3). As shown in Table 3, our results indicated that the mean allele number within the studied taxa were in the order of HB (4.96) > HS(4.75) > HD (3.80) > HH (3.6) .

The PIC values were different within the two species with a mean PIC of 0.639 for *H. vulgare* and 0.316 for *H. bulbosum*. Two primer pairs in HB accessions including Bmag0211 and EBmac0518 had only one allele per locus (160 and 150 bp respectively) but they had eight alleles (from 168 to 200 bp) and three alleles (150 to 165 bp) respectively in HV. The primer EBmatc0040 had two repeated and common band in HV and HB accessions. Mean of allele's number and PIC in HV (7.92 and 0.639 respectively) was higher than HB (4.96and 0.316 respectively).

The mean genetic similarity within HB accessions was 0.755, within HH 0.451, HD 0.433 and HS 0.430. All of the 48 primer pairs tested detected interspecies polymorphisms. Generally, the genetic diversity within the species, subspecies and varieties were in the order HS > HD > HH > HB (Table 3).

CLUSTER ANALYSIS

Cluster analyses showed that the 48 transferred SSR markers can be suitable for the analysis of phylogenetic relationships among *H. vulgare* and *H. bulbosum*. Groupings in dendrogram clearly followed the taxonomic classifications with high bootstrap values (Fig. 2). Accessions were divided into two groups, one including the *H. vulgare*, and the other including the *H. bulbosum* accessions. The *H. vulgare* cluster was subdivided into two sub clusters: one included the *H. vulgare* subsp. *spontaneum* accessions and the other one included *H. vulgare* subsp. *vulgare* with the later divided again into subclusters var. *distichon* and var. *hexastichon*. The *H. murinum* which was included as outgroup in the analysis was placed well away from H genome containing species (Fig. 2).

Discussion

Many studies have indicated that microsatellite primers of a species could be used and amplified in its close relatives (Brown et al. 1990; Hernàndez 2002). The large numbers of microsatellite markers being developed in barley provides a valuable SSR marker resource (Hernandez et al. 2002) which can be exploit in genetic characterization of wild related species. In this study 51.61% of the barley microsatellite primer pairs reproducibly amplified products in *H. bulbosum* and can be used for genetic analysis of this valuable species. Transferability of barley SSRs to *H. bulbosum* in the present study is comparable to those of other studies in the literature. Gupta (2003) has indicated that about 50% SSR primers were transferable from *Triticum* to *Hordeum*. Sharifi Tehrani et al. (2008) reported 75% transferability of *Festuca arundinacea* derived SSRs to *Lolium persicum*. Our findings, thus, confirm that about half of barley SSRs is transferable to *H. bulbosum*. Castillo et al. (2010) reported that from 130 barley genomic microsatellites, 71 (54.6%) SSR primer pairs gave a reliable amplification from *H. chilense* Roem et Schults genome, and 20 (15.4%) of the amplified PCR primers showed polymorphism in the lines used. Tang et al. (2006) showed that 86.8% of wheat derived SSRs produced amplicons in barley, 77.0% in rice and 68.3% in maize. Zhang et al. (2005) reported the transferability of bread wheat EST-SSRs to closely related *Triticeae* species, ranging from 76.7% for *A. tauschii* Cosson to 90.4% for *T. durum* Desf. Lower transferability of barley SSRs to the *Hordeum* species in this study in compare with that reported for bread wheat SSRs indicated that the speciation in the genus *Hordeum* is probably accompanied whit high genomic differentiations. Different level of SSR transferability in different studies may be influenced by the taxa included in the analyses or the SSR markers selected by chance could not reveal the exact transferability level.

Some of the primer pairs that successfully amplified DNA segments in *H. vulgare* failed to amplify product from *H. bulbosum* accessions in this study. This could be due to the divergence in the microsatellite flanking sequences, creating a null allele, or H genome in the *H. bulbosum* have encountered high genomic differentiations since its separation from other H genome species. The results of this study clearly showed that barley microsatellite markers are valuable and cost-effective molecular markers for studying the population structure of *H. bulbosum*. Further analysis of transferred SSRs to *H. murinum* showed very low level of reproducibility and polymorphism among different accessions of this species (data not shown) indicating that this SSRs are not reliable markers for *H. murinum* genetic analysis. Although the allele number in *H. bulbosum* was more than HS, Hd and HH, but the genetic diversity in *H. vulgare* subspecies and varieties was more than *H. bulbosum* (Table 3).

One of the aims of this study was to test efficiency of barley SSRs to infer phylogenetic relationships among the *H. bulbosum*, the cultivated barley and the wild barley. As evidenced in dendrogram (Fig. 2), the clusters were clearly correlated with the taxonomic groups. These results showed that the SSR markers are reliable markers to infer the phylogenetic relationships within the H genome containing species. The rate of transferability across species in this study confirm the general observation that the rate of SSRs transferred across species decay as the species are more

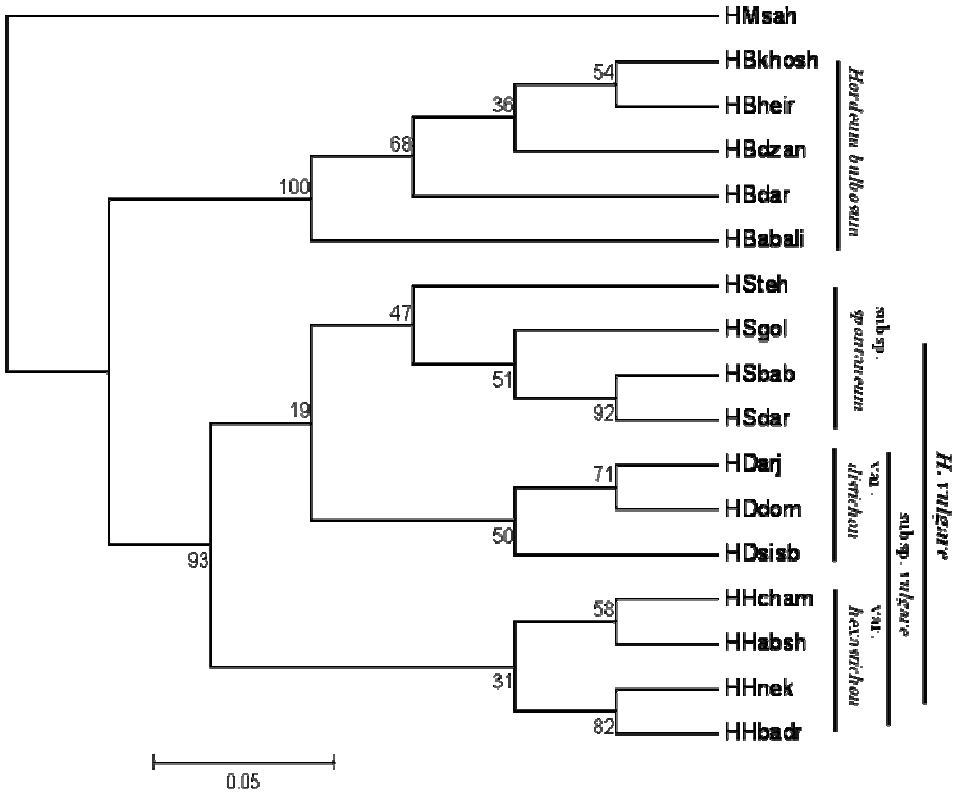

Fig. 2. A UPGMA dendrogram showing relationships between HB (*Hordeum bulbosum*), HS (*H. vulgare* subsp. *spontaneum*), HD (H. *vulgare* subsp. *vulgare* var. *distichon*) and HH (*H. vulgare* subsp. *vulgare* var. *hexastichon*) (see table 1), based on the 48 barley microsatellites (see table 3). HM (*H. murinum*) was treated as outgroup. The bootstrap values are shown on branches.

phylogenetically distant, that is in agree with Varshney et al. (2005). The secondary gene pool, *H. bulbosum,* occupied an isolated position intermediate between the primary and tertiary gene pool (*H. murinum*), with high level of genetic distance, that is in agree with Terzi et al. 2001. Data obtained from cluster analysis were in complete agreement with taxonomic classifications proposed previously based on comparisons of morphological, cytological and reproductive characters (von Bothmer et al. 1995, Terzi et al. 2001, Komatsuda et al. 1999, Kakeda et al. 2009). This can be interpreted as reliability of barley SSRs for evaluating phylogenetic relationships among the studied taxa.

Conclusion

Our study showed the transferability of some barley SSRs from *H. vulgare* to *H. bulbosum* with high level of polymorphism within this species, which can be used for the genetic analysis of *H. bulbosum*. High polymorphism rates despite the limited number of genotypes tested, indicated that these SSR markers can be used in study of genetic diversity, gene mapping and marker assisted selection studies in H genome containing species of the genus *Hordeum*. The transferred markers have shown to be useful for phylogenetic studies within this group. The availability of additional sets of mapped SSR markers for barley and other *Hordeum* genomes will assist the development of molecular maps for *H. bulbosum* and its integration into the genomic network of grass species.

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References

Alves Rafael M., Sebbenn A. M., Artero A. S. & Figueira A. 2006: Microsatellite loci transferability from Theobroma cacao to Theobroma grandiflorum. -Molecular Ecology Notes. 6: 1219–1221.

Anderson J. A., Churchill G. A., Autrique J. E., Tanksley S. D. & Sorrells M. E. 1992: Optimizing parental selection for genetic linkage maps. -Genome 36: 181-186.

Asfaw Z. & Bothmer R. 1990: Hybridization between landrace varieties of Ethiopian barley (Hordeum vulgare ssp. vulgare) and the progenitor of barley (H. vulgare ssp. spontaneum). -Hereditas 112: 57–64.

Bothmer R., Jacobson N., Baden C, Jorgensen R. B. & Linde-Laursen I. 1995: An ecographical study of the genus Hordeum. 2nd Ed. -International Plant Genetic Resources Institute, Rome, Italy. pp 1-124.

Bravo J. P., Akemi H. A., Lara C. M., Angelici C. D., Lopes C. R. & Gimenes M. A. 2006: Transferability and use of microsatellite markers for the genetic analysis of the germplasm of some Arachis section species of the genus Arachis. -Genet Mol Biol 29(3): 516-524.

Brown A. D. H., Burdon J. J., Grace J. P. 1990: Genetic structure of Glycine canescens, a perennial relative of soybean. -Theoretical and Applied Genetics 79: 729-736.

Castillo A., Budak H., Martin A. C., Dorado G., Borner A., Roder M. & Hernandez P. 2010: Interspecies and intergenus transferability of barley and wheat D-genome microsatellite markers. -Annales of Applied Biology 156: 347–356.

Castillo A., Budak H., Varshney R. K., Dorado G., Graner A. & Hernandez P. 2008: Transferability and polymorphism of barley EST-SSR markers used for phylogenetic analysis in Hordeum chilense**.** -BMC Plant Biology 8:97 doi:10.1186/1471-2229-8-97.

Dice L. R. 1945: Measures of the amount of ecologic association between species. -Ecology 26: 297-302.

Ellis, J. R. & Burke J. M. 2007: EST-SSRs as a resource for population genetic analysis. -Heredity 99: 125-132.

Fahima T., Roder S., Grama A., Nevo E. 1998: Microsatellite DNA polymorphism divergence in Triticum dicoccoides accessions highly resistant to yellow rust. -Theor Appl Genet 96: 187-195.

Gimenes Marcos A., Hoshino A. A., Barbosa A. V. G., Palmieri D. A. & Lopes C. R. 2007: Characterization and transferability of microsatellite markers of the cultivated peanut (Arachis hypogaea). -BMC Plant Biol 7:9 doi:10.1186/1471-2229-7-9.

Gupta P. K., Rustgi S., Sharma S., Singh R., Kumar N. & Balyan H. S. 2003: Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. -Mol Genet Genomics 270(4): 315-323.

Hernández P., Laurie D. A., Martín A. & Snape J. W. 2002: Utility of barley and wheat simple sequence repeat (SSR) markers for genetic analysis of Hordeum chilense and tritordeum. -Theoretical and Applied Genetics 104:735–739.

Jenabi T., Saeidi H. & Rahiminejad M. R. 2011: Biodiversity of Secale strictum in Iran measured using microsatellites. -Gentics Resource Crop Evolution 58 (4): 497-505.

Kakeda K., Taketa S. & Komatsuda T. 2009: Molecular phylogeny of the genus Hordeum using thioredoxin-like gene sequences. -Breeding Science 59: 595-601.

Kasha K. J., Pickering R. A., William H. M., Hill A., Oro R., Reader S. & Snape J.W.1996: GISH and RFLP facilitated identification of a barley chromosome carrying powdery mildew resistance from Hordeum bulbosum. -Proc. 7th Intl. Barley Genet. Symposium. University Extension Press, University of Saskatchewan 1:.338-340.

Komatsuda T., Nakamura I., Takaiwa F. & Oka S. 1998: Development of STS markers closely linked to the vrs1 locus in barley, Hordeum vulgare. -Genome 41: 680–685.

Liu Z. W., Biashev R. M. & Saghai Maroof M. A. 1996: Development of simple sequence reapeat DNA marker and their integration into a barley linkage map. -Theoretical and Apllied Genetics 93: 869-876.

Martin A., Martin L. M., Cabrera A., Ramirez M. C., Gimenez M. J., Rubiales D., Hernandez P. & Ballesteros J. 1998: The potential of Hordeum chilense in breeding Triticeae species. In: Jaradat AA, Enfield NH (eds) Triticeae III. -Science Publishers, pp. 377–386.

Pickering R. A. 1992: Monosomic and double monosomic substitutions of Hordeum bulbosum L. chromosomes into H. vulgare L. -Theoretical and Apllied Genetics 84: 466-472.

Pickering R. A., Malyshev S., Künzel G., Johnston P. A., Korzun V., Menke M. & Schubert I. 2000: Locating introgressions of Hordeum bulbosum chromatin within the H. vulgare genome. -Theoretical and Apllied Genetics 100: 27-31.

Ramsay L., Macaulay M., Degli Ivanissevich S., Maclean K., Fuller J., Edwards K. J., Tuvesson S., Morgante M., Massari A., Maestri E., Marmiroli N.,

Sjakste T., Ganal M., Powell W., Waugh R. 2000: A simple sequence repeat-based linkage map of barley. -Genetics 156: 1997-2005.

Rolf F. J. 2000: NTSYS-PC: Numerical Taxonomy and Multivariate Analysis System. Ver. 2.1., Exter Software, Setauket, New York, ISBN: 0925031-30-5, pp: 1-38.

Ruge B., Michel M. & Pickering R. A., Proeseler G. & Wehling P. 2000: Gene introgressions from H. bulbosum into cultivated barley cause resistance to different pathogens. -Plant and Animal Genome Conference VIII, 09-12.01.2000. San Diego, CA, USA.

Sharifi Tehrani M., Mardi M., Saeidi H., Gharehyazi B. & Assadi M. 2008:Transferability of genomic and EST-Microsatellites from Festuca arundinacea Scherb. to Lolium persicum Boiss. and Hohen. ex Boiss. -International Journal Botany 4 (4): 476-480.

Taketa S., Ando H., Takeda K. & von Bothmer R. 1999: Detection of Hordeum marinum genome in three polyploid Hordeum species and cytotypes by genomic in situ hybridization. -Hereditas 130: 185–188.

Taketa S., Ando H., Takeda K. & von Bothmer R. 2001: Physical locations of 5S and 18S–25S rDNA in Asian and American diploid Hordeum species with the I genome. -Heredity 86: 522–530.

Taketa S., Hirotaka A., Takeda K., Ichii M. & von Bothmer R. 2005: Ancestry of American Polyploid Hordeum Species with the I Genome Inferred from 5S and 18S–25S rDNA. -Annals of Botany 96: 23–33.

Tang J., Gao L., Cao Y. & Jia J. 2006: Homologous analysis of SSR-ESTs and transferability of wheat SSR-EST markers across barley, rice and maize. -Euphytica 151**:**87-93.

Terzi V., Pecchioni N., Faccioli P., Kučera L. & Stanca A. M. 2001: Phyletic relationships within the genus Hordeum using PCR-based markers. -Geneticc Resource Crop Evolution 48 (5): 447-458.

Varshney R. K., Graner A. & Sorrells M. E. 2005: Genic microsatellite markers in plants: features and applications. -Trends Biotechnology 23:48-55.

Wang D., Shi J., Carlson S. R., Cregan P. B., Ward R. W. & Diers B.W. 2003: A Low-Cost, High-Throughput Polyacrylamide Gel Electrophoresis System for Genotyping with Microsatellite DNA Markers. - Crop Science 43:1828–1832

Zhang L.Y., Bernard M., Leroy P., Feuillet C. & Sourdille P. 2005: High transferability of bread wheat EST-derived SSRs to other cereals. -Theoretical Applied Genetics 111:677-687**.**