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Study of the genetic diversity of Korean, Chinese and Japanese landraces of barley (*Hordeum vulgare* L.) using microsatellites

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Abstract: Barley (*Hordeum vulgare* L.) is one of important winter cereals in the world and has been the subject of numerous genetic investigations. Studies of its genetic diversity based on germplasm have a significant impact on crop breeding and conservation of genetic resources. This study was conducted to reveal the genetic diversity in barley landraces from Korean, Chinese and Japanese populations and evaluate the discrimination ability of SSR markers, distributed uniformly throughout the barley genome. Seven SSR primers were used to screen a set of 737 diverse barley landraces from Korea, China and Japan. The observed number of alleles per locus (Na), the effective number of alleles (Ne), and the mean gene diversity (He) were 11.14, 3.6245 and 0.7048, respectively. The number of alleles per locus was highest in Chinese landraces (8.9 alleles), followed by Korean (8.6) and Japanese (6.4). In this regard, HVKASI primer may be useful to distinguish Japanese landraces from others, as this unique allele was only detected at 175 bp in Japanese landraces. The average value of genetic distance was D=0.935. The largest genetic distance (D=1.209) among the three regional (representing each country in general) populations was found between Korean and Japanese populations. Based on the UPGMA dendrogram, four major groups can be distinguished at the similarity value of 0.43. The scatter plot showed overlapping in the central part amongst 3 groups of barley landraces. SSR markers are a powerful tool to examine functional diversity. Rich barley gene pool can be very useful for meeting current and future challenges in barley raising.

Key words: barley, genetic diversity, landraces, SSR markers

1. Introduction

Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop, after wheat, maize and rice. It is a diploid (2n=2x=14), largely self-fertilizing species with a large genome of $5.3x10^9$bp/1C. The genetic advantages of working with a self-compatible true diploid have resulted in barley being proposed as a model for the entire Triticeae. It was one of the earliest domesticated crops and has been cultivated since the beginnings of civilization. The antiquity of barley is documented from grains, or fragments of grains and husks, uncovered in archaeological excavations widely scattered in the Near and Middle East. Many of these fragments were carbon dated to the period from 7,000 to 5,000 B.C. (Clark 1967; Harlan 1979). Barley is grown over a broader environmental range than any other cereal and has persisted as a major cereal crop over so many centuries because of its wide ecological adaptation, usefulness as a feed and food grain, and superiority of barley malt for use in brewing (Poehlman 1985).

Barley is a genetically well-studied crop. Cultivated barley, *Hordeum vulgare*, including its wild relative *H. vulgare* subsp. *spontaneum*, belongs to the primary gene pool of *Hordeum* (Plucknett *et al*. 1987). Genetic diversity is of fundamental importance for genetic improvement and knowledge of germplasm diversity has a significant impact on the improvement of crop plants. Due to modern raising, it has been suggested

that genetic diversity in barley has been continuously decreasing. Narrow genetic diversity is a problem in raising as it reduces adaptation to biotic and abiotic stresses. Therefore, it is necessary to investigate genetic diversity in barley germplasm to increase genetic variation for future barley breeding (Huang *et al*. 2002). Assessment of genetic variability between individuals and regional populations has been based on the analysis of pedigree records, morphological traits and molecular markers. However, pedigree data do not account for the effect of selection, mutation and random genetic drift. Use of morphological traits for a plant diversity analysis has been criticized because genetic control is largely unknown and expression depends on environmental factors. Nonetheless, DNA-markers are being successfully used for the advantage of being generally independent of phenotype and, if representative of the entire genome, can provide a comprehensive survey of genetic variation present in a sample of cultivar (Strelchenko *et al*. 1996).

Though various researchers have studied the extent of genetic diversity in barley employing RFLPs (Saghai Maroof *et al*. 1994; Lin *et al*. 1996), RAPDs (Dawson *et al*. 1993), SSRs (Saghai Maroof *et al*. 1994; Lin *et al*. 1996), and AFLPs (Pakniyat *et al*. 1997), to the best of our knowledge no report is available describing the diversity in barley landraces in Korea, Japan and China. This study was therefore conducted to reveal the genetic diversity of microsatellites in the populations of Korean, Chinese and Japanese barley landraces to compare SSR diversity in these countries, and to make good use of fundamental data of sovereign rights under the Convention on Biological Diversity (CBD).

2. Materials and methods

2.1. Plant materials

A total of 737 accessions of barley (*Hordeum vulgare* L.) from three sets of experimental materials were examined. One set of 452 accessions of Korean barley landraces was acquired from the Okayama University in Japan (http://www.rib.okayama-u.ac.jp/barley). The other sets included 104 Japanese accessions and 181 accessions of Chinese landraces coming from the world barley collection of the United States Department of Agriculture. These materials originated from different

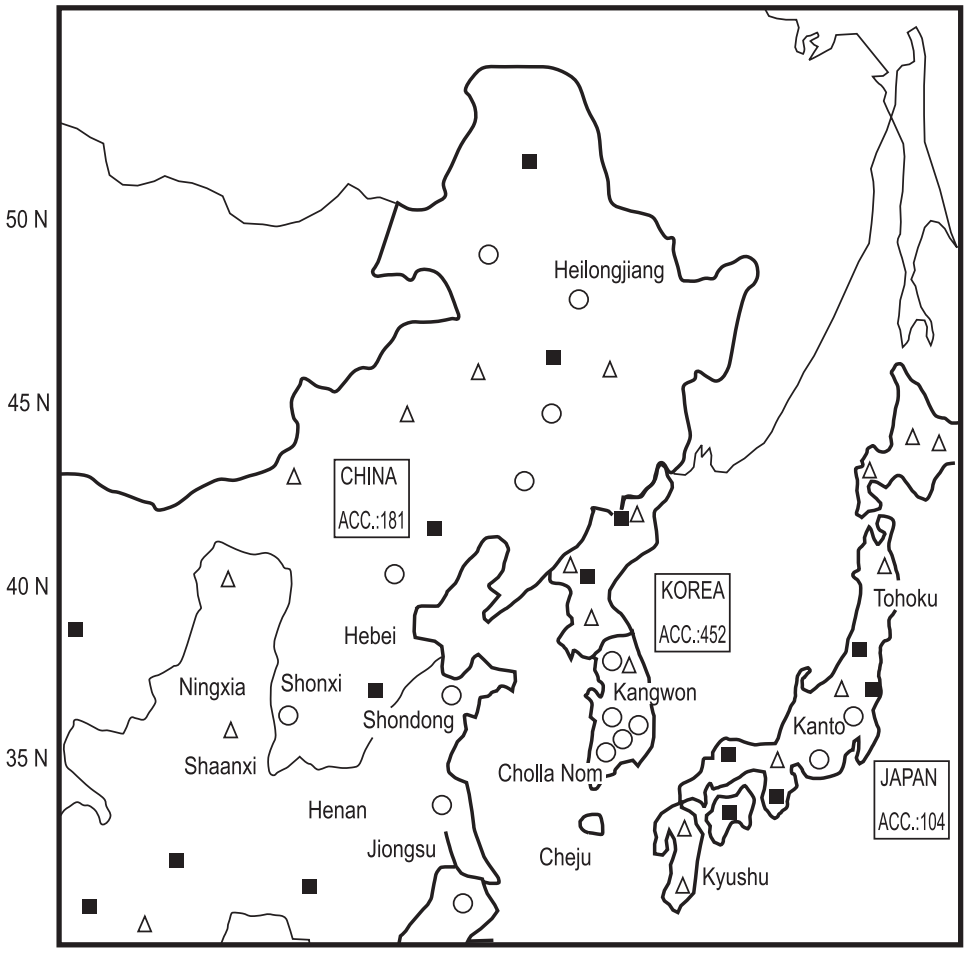

Fig. 1. Geographic location of barley landraces collection sites in Korea, China and Japan
Explanations: Δ – 1~5 Acc., ■ – 6~10 Acc., ○ – 11~ Acc.

longitudes and latitudes across the species ecological spectrum in these three countries (Fig. 1).

2.2. DNA isolation and PCR amplification

Barley seeds were germinated in Petri dishes at room temperature after incubation at 4˚C. Genomic DNA was extracted from fresh leaves of 10-day old plants of each accession using Dneasy Plant Mini Kits (Qiagen #69106). Seven SSR loci with mono- or dinucleotide repeats were selected for the study. The SSR loci were chosen based on their length polymorphism and they represented one marker from each chromosome. Forward primers of the 7 SSR loci were fluorescent labeled (Table 1). The PCR reaction was performed in a 20 µl volume using a DNA Engine DYAD (MJ Research). The reaction mixture contained 250 nM of each primer, 0.25 nM of dNTP, 1X PCR buffer containing 50 mM KCl, 10 mM TRIS-HCl pH 9.0, 0.1% Triton X-100, 1U of Taq-polymerase and 20 ng of template DNA. PCR conditions were optimized for each primer pair and depended on the primer pairs used; amplification of the SSRs was performed using one of the following three sets of PCR conditions: (*i*) 'Touchdown' PCR consisting of 18 cycles at 94˚C for 1 min denaturation, for 30 s anniling and 72˚C for 1 min extension. Annealing temperatures were progressively decreased by 1˚C every second cycle from 64˚C to 55˚C. The PCR program for 30 additional cycles was 94˚C for 1 min, 55˚C for 1min and 72˚C for 1min. The reaction was ended with a final 5 min extension at 72˚C; (*ii*) regular PCR profile consisting of one cycle at 94˚C for 3 min, 55˚C for 2 min and 72˚C for 1.5 min, followed by 30 cycles each at 94˚C for 1 min, 55˚C for 2 min and 72˚C for 1.5 min; (*iii*) the third set of PCR conditions was the same as reported by Becker and Heum (1995) and Röder *et al*. (1998).

2.3. Fragment analysis

For PCR fragment-size determinations, amplified products from SSR loci with different fluorescent label were simultaneously analyzed in automated DNA sequencer. Therefore, after PCR, 2.0 µl of each FAM-labeled, HEX-labeled, and NED-labeled PCR product were combined. 0.2 µl of internal size standard (Genscan 500, ROX) was mixed with 1µl of the PCR product and 11 µl of formamide. PCR fragment sizes of SSR loci were read using the GeneScane 3.7 software (Applied Biosystems). Allele sizes were determined using the genotyper 2.1 software (Applied Biosystems). One primer from each pair was resynthesized with a FAM, HEX, or NED 5' end-labeled nucleotide to allow data capture on an automated DNA sequencer. Each primer pair used to screen the reference set for allelic variants was easily and reliably obtained. The characteristic stuttering of dinucleotide SSR-base markers was clearly evident.

2.4. Statistical analysis

The similarity matrix was analyzed by an unweighted pair group method with the arithmetic mean (UPGMA) procedure using the NTSYS software to create a dendrogram representing diversity among Korean, Chinese and Japanese barley landraces. Fragments amplified by microsatellite primers were scored as present (1) or absent (0). The genetic similarities (GS) were calculated for each pair of lines using the Dice's similarity index (Dice 1945). The genetic variation of each locus was measured in terms of the number of alleles and the observed gene diversity (Nei 1978) by using the formula: H (gene diversity) = $1-\Sigma^{n}P_{i}^{2}$, where P_{i} is the frequency of the i^{th} allele, and n is the number of samples. Anderson *et al*. (1993) referred to gene diversity as the polymorphic information content (PIC). Data were entered in the form of single-individual genotypes. The diversity value for the total sample was further partitioned into their respective components as described by Nei (1973). Calculations were performed using POPGENE version 1.3 (Yeh *et al*. 1999). Similarity coefficients were also used for the cluster analysis of varieties, using the ,Shan' subprogram of NTSYS-PC (version 2.01, Exeter Software, Setauket, NY, USA; Rohlf 1997) and to build dendrograms by the UPGMA. Principle coordinate analysis was also conducted to show multiple dimensions of the distribution of genotypes in a scatter-plot (Keim *et al*. 1992).

3. Results

3.1. Automated sizing of SSR loci

Fluorescent labeled PCR products from 7 SSR loci were analyzed in each peak (Fig. 2). Three of the loci, BMS64 with (AC)n, HVM20 and HVM14, both with (GA)n repeats, were labeled with FAM(blue), 2 others, HVBKASI and HVM68, with (C)n and (GA)n repeats, respectively, were labeled with HEX(yellow), and the last 2 loci, HVM6 and HVM60, with (GA)n and (AG)n(GA)n, respectively, were labeled with NED (green) (Table 1).

3.2. Distribution of alleles at polymorphic SSR loci

The studied regional populations of barley landraces and SSR loci were polymorphic when considered in general. A total of 78 amplified fragments were detected at 7 loci. A summary of genetic diversity data for 737 accessions is given in Table 2. For each primer we calculated the total number of alleles per locus, the effective number of alleles, and mean gene diversity (Nei 1973). The average values were: Na=11.14 (range 6-19); Ne=3.6245 (range 2.6184-5.492), Ht=0.7048 (range=0.6181-0.8179).

The number of alleles per locus was highest among Chinese landraces (8.9 alleles), followed by Korean

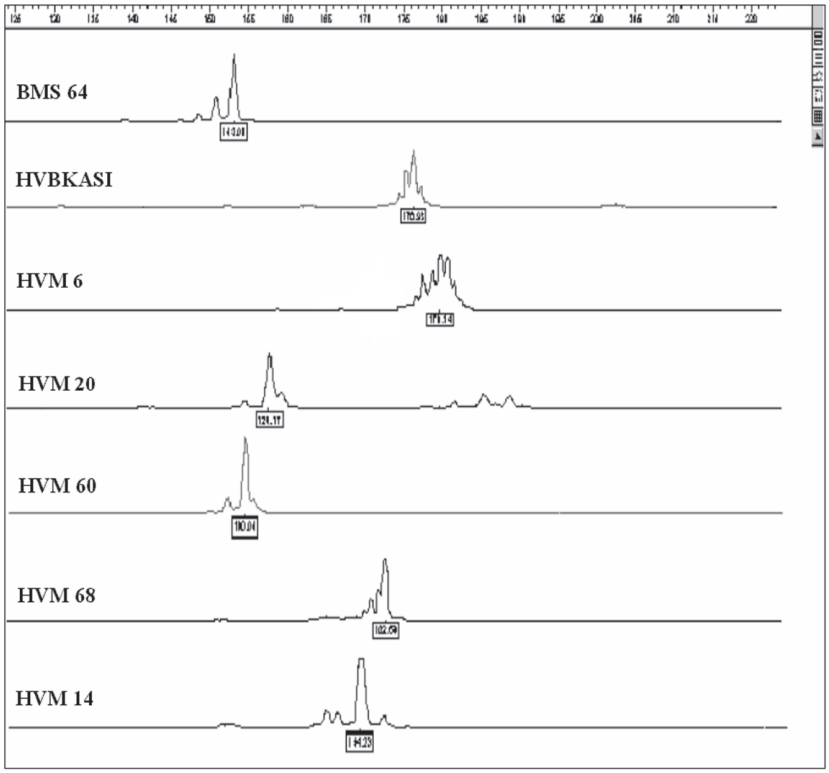

Fig. 2. Electropherogram of alleles scored at all studied loci by ABI prism 3100 Genetic Analyzer

Table 1. Microsatellite primers, core motifs and number of repeats, chromosomal location and reaction details used in PCR assays

Primer	Primer Sequences(5' to 3')	Repeats	Chromosome	Fluorescent Label†	PCR‡
BMS64	F-ACACCTTCCCAGGACAATCCATTG R-AGCACGCAGAGCACCGAAAAAGTC	$(AC)_{21}$	1(7H)	FAM	2
HVBKASI	F-ATTGGCGTGACCGATATTTATGTTCA R-CAAAACTGCAGCTAAGCAGGGGAACA	$(C)_{10}$	2(2H)	HEX	3
HVM6	F-CATGAATGAATGATTGGTTTTG R-CGCATCCGTATGTATGAGTAA	$(GA)_9$	7(5H)	NED	1
HVM20	F-CTCCACGAATCTCTGCACAA R-CACCGCCTCCTCTTTCAC	$(GA)_{19}$	5(1H)	FAM	1
HVM68	F-AGGACCGGATGTTCATAACG R-CAAATCTTCCAGCGAGGCT	$(GA)_{22}$	4(4H)	HEX	2
HVM60	F-CAATGATGCGGTGAACTTTG R-CCTCGGATCTATGGGTCCTT	$(AG)_{11,}(GA)_{14}$	3(3H)	NED	1
HVM14	F-CGATCAAGGACATTTGGGTAAT R-AACTCTTCGGGTTCAACCAATA	$(GA)_{11}$	6(6H)	FAM	3

Explanations: microsatellite primer sequences are based on the experiment reported in this manuscript and have not been taken from an external source; chromosome numbering follows barley nomenclature with the homologous chromosome group given in parenthesis (IBGS, 1996); ‡ – The number represent one of three PCR conditions used in this study; †FAM – Blue, NED – Green, HEX – Yellow

Table 2. Observed number of alleles (Na), effective number of alleles (Ne), and mean gene diversity (Ht) from the 7 microsatellite markers

Locus	Accession	Na	Ne	Ht
BMS64	737	8	3.0394	0.6710
HVKASI	737	12	4.1402	0.7585
HVM6	737	9	2.8829	0.6531
HVM20	737	12	4.4030	0.7729
HVM68	737	19	2.7963	0.6424
HVM60	737	12	5.4920	0.8179
HVM14	737	6	2.6184	0.6181
MEAN	737	11.14	3.6245	0.7048
S.D.		4.18	1.0700	0.0800

(8.6) and Japaneses (6.4). The number of region specific alleles and number of accessions carrying rare alleles were highest among Korean landraces with 13 specific alleles and 66 accessions carrying rare alleles. Japanese barley landraces had 2 specific alleles and 3 accessions carrying rare alleles (Table 3). These results suggest that largest genetic variation, based on the number of total alleles and number of alleles per locus, is among Chinese landraces. We compared the number of alleles of the 7-shared SSR loci in Korean, Chinese and Japanese populations (Table 4). Of the total of 78 alleles at 7-shared loci, 60 (76.9%) were observed in

Table 3. Comparison of genetic diversity among Korean, Chinese and Japanese landrace barley accessions

Collection site	Korea	China	Japan	Mean
No. of accessions	452	181	104	
No. of total alleles	60	62	45	55.67
No. of alleles per locus	8.6	8.9	6.4	7.97
No. of region-specific alleles	13	6	2	7
No. of accessions carrying rare alleles	66	22	3	30.33
Mean P.I.C. value	0.569	0.575	0.375	0.506

Table 4. Allele frequencies at 7 loci in Korean, Chinese and Japanese landrace barley accessions

Primer	Allele (bp)	Korea (n=452)	China (n=181)	Japan (n=104)	Total (n=737)
BMS64	139		0.04	0.02	0.01
	141			0.02	0.00
	151	0.00	0.03	0.08	0.02
	153	0.70	0.80	0.74	0.73
	155	0.01	0.09	0.03	0.03
	157		0.03		0.01
	167	0.28	0.01	0.12	0.19
	169	0.00			0.00
HVBKASI	175	0.05	0.12	1.00	0.21
	176	0.42	0.46		0.40
	177	0.17	0.16		0.15
	178	0.14	0.02		0.10
	179	0.03	0.03		0.02
	180	0.07			0.04
	181	0.02			0.02
	182	0.00			0.00
	183	0.00			0.00
	195	0.00			0.00
	196	0.02	0.10		0.04
	197	0.04	0.05		0.01
HVM6	171	0.01	0.01	0.02	0.01
	173		0.02	0.01	0.01
	175	0.01	0.03		0.01
	177	0.25	0.11	0.13	0.20
	179	0.66	0.77	0.50	0.66
	181	0.05	0.03	0.25	0.08
	183	0.01	0.02	0.10	0.02
	195	0.00			0.00
	197	0.02			0.01
HVM20	124	0.00	0.03		0.01
	126	0.05	0.10	0.01	0.06
	128	0.48	0.45	0.65	0.49
	130		0.01		0.00
	138	0.00			0.00
	140		0.01	0.01	0.00
	142	0.01	0.02		0.01
	144	0.03	0.02	0.05	0.03
	146	0.15	0.13	0.16	0.14
	148	0.22	0.14	0.08	0.18
	150	0.06	0.10	0.08	0.07
	152	0.00	0.03		0.01
HVM68	178	0.00			0.00
	180	0.8	0.75	0.83	0.79
	182	0.06	0.06	0.02	0.05
	184	0.00	0.01		0.01
	188		0.04	0.05	0.02
	190	0.00			0.00
	192		0.02		0.00
	196		0.04	0.02	0.01
	198	0.00	0.01		0.00
	200	0.00	0.04		0.01
	202	0.01	0.01	0.01	0.01
	204	0.01	0.01		0.01
	206	0.1	0.01	0.04	0.07
	210	0.00	0.01	0.02	0.01

Primer	Allele (bp)	Korea (n=452)	China (n=181)	Japan (n=104)	Total (n=737)
	212		0.01		0.00
	214			0.01	0.00
	218	0.00			0.00
	222		0.01		0.00
	238	0.00			0.00
HVM60	79	0.08	0.25	0.20	0.14
	81	0.07	0.02	0.04	0.05
	97		0.01	0.01	0
	99	0.19	0.05	0.09	0.14
	101	0.44	0.37	0.45	0.42
	103	0.15	0.11	0.04	0.12
	105	0.03		0.01	0.02
	107	0.04	0.02	0.02	0.03
	109		0.03	0.02	0.01
	111	0.02	0.01	0.05	0.02
	113	0.02	0.10	0.03	0.04
	115		0.04	0.03	0.01
HVM14	152	0.01	0.01		0.01
	154	0.42	0.42	0.62	0.45
	156	0.54	0.48	0.36	0.50
	158	0.02	0.09	0.01	0.04
	160	0.01	0.01	0.01	0.01
	162		0.01	0.01	0.00

the Korean population, 62 (79.5%) in Chinese population, while only 45 (57.6%) in Japanese population. The number of unique alleles in Korean populations (13) was much higher than in Chinese (6) and Japanese (2) populations. Description of 7 SSR loci included product size range, PIC values and main product size for each

Table 5. Description of 7 barley microsatellites used, including range of product sizes, PIC value and main product size in Korean, Chinese and Japanese landrace barley groups

Primer	Chromosomal location	Countries	Product range (bp)	Main product size(bp)	PIC
BMS 64	1(7H)	Korea	151-169	153	0.464
		China	139-167	153	0.342
		Japan	139-167	153	0.472
		Total	139-169	153	0.426
HVBKASI	2(2H)	Korea	175-196	176	0.759
		China	175-197	176	0.746
		Japan	175	175	0
		Total	175-197	176	0.502
HVM6	7(5H)	Korea	171-197	179	0.506
		China	171-183	179	0.399
		Japan	171-183	179	0.668
		Total	171-197	179	0.524
HVM20	5(1H)	Korea	124-152	128	0.695
		China	124-152	128	0.746
		Japan	126-150	128	0.562
		Total	124-152	128	0.668
HVM68	4(4H)	Korea	178-238	180	0.288
		China	180-222	180	0.436
		Japan	180-214	180	0.272
		Total	178-238	180	0.332
HVM60	3(3H)	Korea	79-113	101	0.744
		China	79-115	101	0.768
		Japan	79-115	101	0.719
		Total	79-115	101	0.744
HVM14	6(6H)	Korea	152-160	156	0.530
		China	152-160	156	0.590
		Japan	154-162	154	0.494
		Total	152-162	156	0.538
	Mean	Korea			0.569
		China			0.575
		Japan			0.375
		Total			0.506

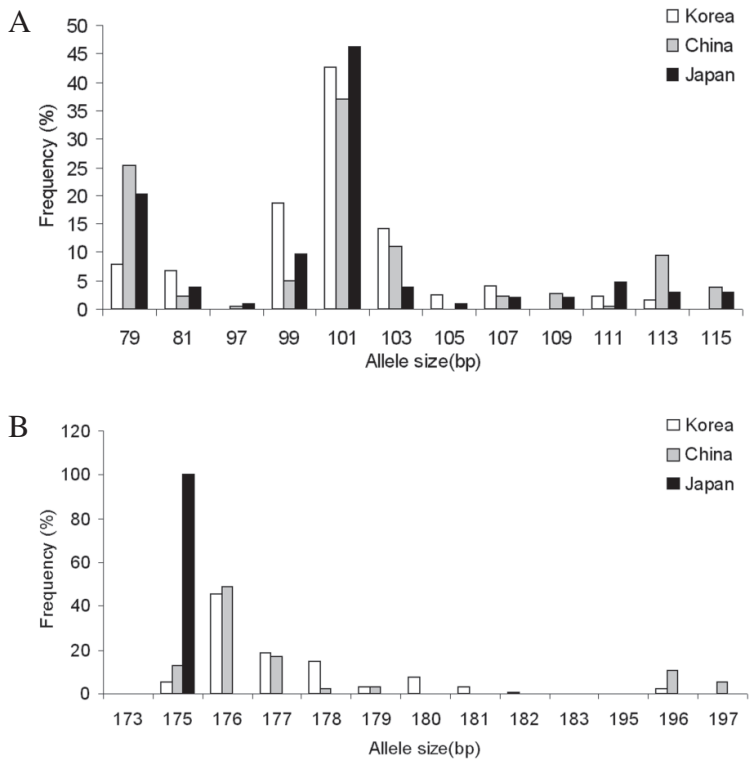

Fig. 3. The distribution of allele frequencies among Korean, Chinese and Japanese barley landraces at the HBVKASI (A) and HVM60 (B) loci

country's barley landrace group (Table 5). The PIC values ranged from 0 (Japan) at the primer HVKASI to 0.768 (China) at the primer HVM60. The average PIC value was highest at HVM60 (0.744) and lowest at HVM68 (0.332). Except for the primer HVKASI from Japanese barley landraces, other showed PIC range from 0.272-0.768. The average PIC value was highest in Chinese landraces (0.575) and those of Korean and Japanese landraces were 0.569 and 0.375, respectively. The distribution of allele frequencies among Korean, Chinese and Japanese barley landraces at the HVKASI and HVM60 primers were shown in Figure 3. The obtained results indicate that the HVKASI primer can be useful to distinguish Japanese landraces from others, because this unique allele was detected only at 175bp in Japanese landraces. On the other hand, the distribution of allele patterns detected in Chinese and Korean landraces was highest at 176bp. Generally, the high degree of polymorphism of SSR markers allows to carry out a rapid and efficient identification of barley genotypes. The allele frequency distribution of HVM60 primer showed a pattern typical of other primers used in this study. It was highest at 101bp among Korean, Chinese and Japanese barley landraces.

Table 6. Genetic distance between Korean, Chinese and Japanese barley landraces regional populations

Country	Korea	China	Japan
Korea	***		
China	0.8487	***	
Japan	1.2087	0.7487	***

3.3. Genetic distance and cluster analysis

The genetic distances (D) among the three regional populations were estimated by unbiased genetic distance (Table 6). The average value was D=0.935 (range 0.749-1.209). The genetic distance between Korean and Japanese populations was largest (D=1.209), whereas between Japanese and Chinese was smallest (D=0.749). The materials used in this study were collected between latitudes 25˚ and 50˚N. This result indicates that differences in the mean genetic distance among three countries are significant. It is postulated that germplasm is more likely to change east-west along the same latitude than north-south across the different latitudes. Because the territory of Korea is smaller than of China and Japan, there is a relationship among aceessions from above 40˚ latitude and below 33˚ in Japan and China. The size of Korean germplasm (452 accessions) is much larger than Chinease (181) and Japanese (104). The results of UPGMA cluster analysis based on SSR genetic similarity matrix are presented in a phenogram (Fig. 4). The

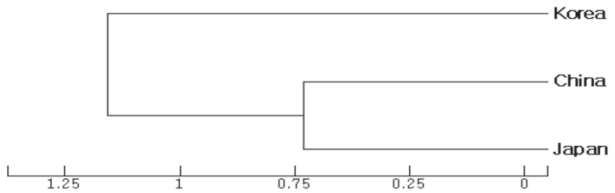

Fig. 4. UPGMA phenogram based on genetic distances, showing the clustering of barley landraces from three studied countries

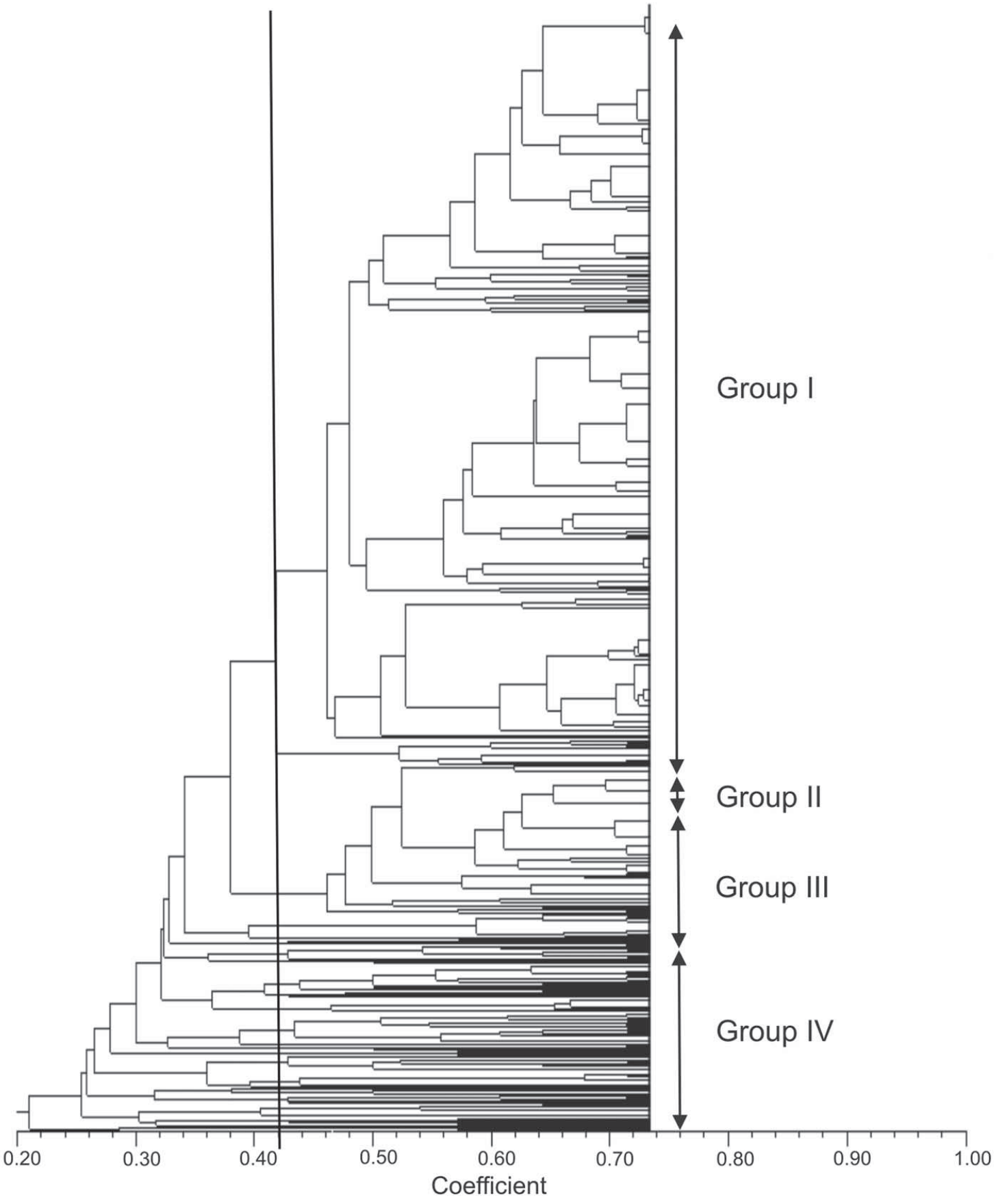

Fig. 5. UPGMA dendrogram based on the Jaccard similarity ratio using 7 microsatellite markers in the 737 barley landraces

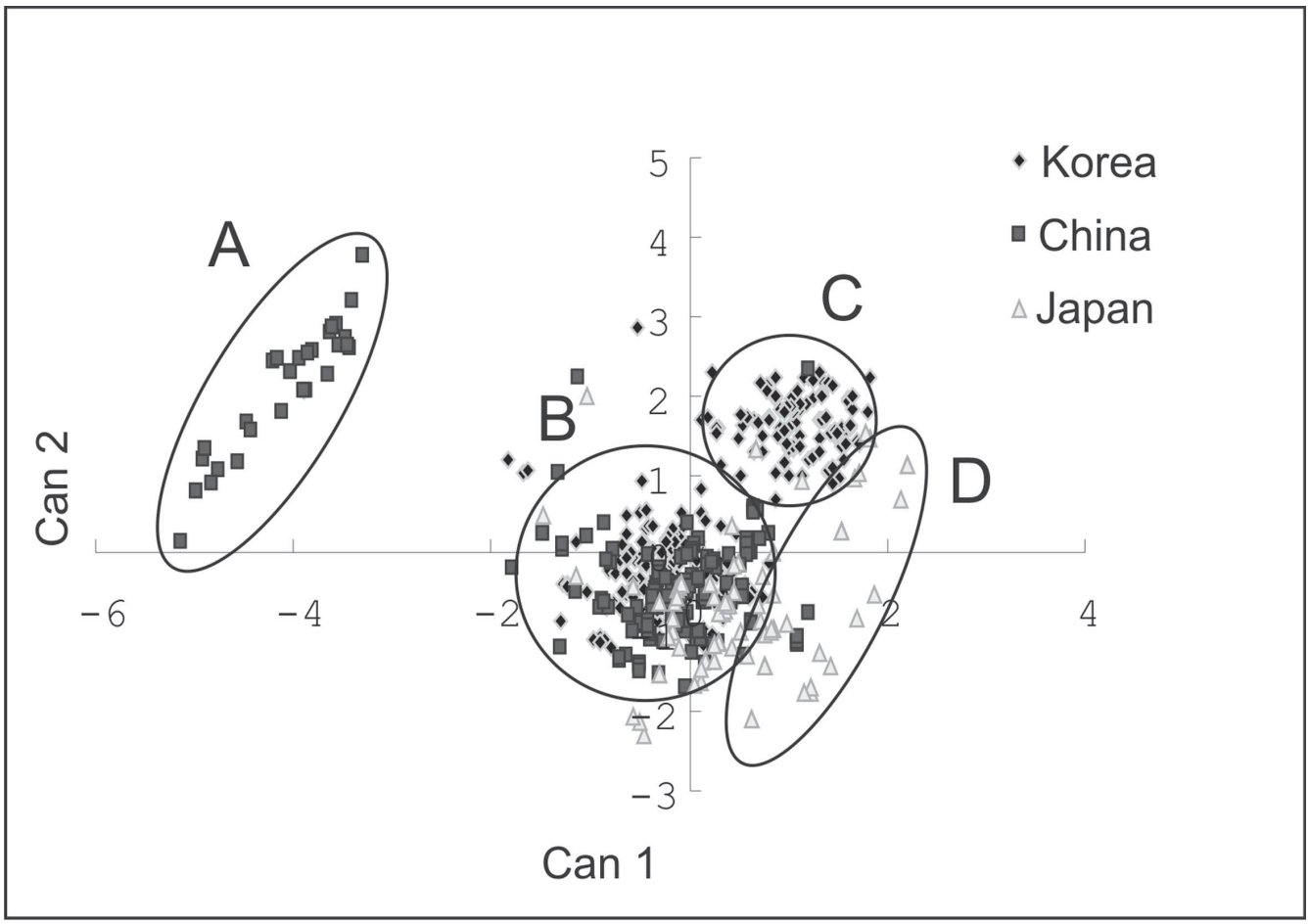

Fig. 6. Canonical discriminant analysis of barley landraces

cluster analysis allowed to identify the genetic relationship of barley genotypes examined in each set and demonstrated a potential and usefulness of SSR markers for a genome analysis in barley. The lowest genetic distances were found between genotypes of the same origin in all three regional populations (Fig. 5). The genetic similarity coefficient for all accessions ranged from 0.21 to 1.00. Four major groups can be distinguished by truncating a dendrogram at similarity value of 0.43. The first group was composed of 283 Korean, 125 Chinese and 70 Japanese landraces, indicating that 64.9% of accessions were included in this group. The second group was composed of 12 Korean, 4 Chinese and one Japanese landrace of barley, the third consisted of 85 Korean, one Chinese and 12 Japanese barley landraces and the fourth of 72, 51 and 21, respectively.

3.4. Canonical discriminant analysis

Canonical discriminant analysis describes portions of genetic variance in a data set (Fig. 6). The scatter plot was separated on the basis of geographic origin of the accessions. This analysis reveals correlations with phenotypic traits, such as hull features, and ecological adaptations, such as latitude, longitude and sowing season. Four groups were distinguished: A, B, C and D, with 29, 604, 86 and 21 accessions, respectively. The group B showed overlapping of accessions from all three countries in the central part. This group was mostly clustered within the group I (Fig. 5). Among Chinese accessions, 29 landraces, grouped in the group A (Fig. 6), show negative values in relation to Can 1 and are clearly distinct from other accessions.

3.5. Genetic differentiation (G_{ST}) within and between regional populations

The total gene diversity (H_T) of populations can be portioned into the mean gene diversity within populations (H_S), the average gene diversity among populations ($D_{ST}= H_T- H_S$) and gene diversity between populations, relative to H_T ($G_{ST}=D_{ST}/H_T$) (Nei 1973). The overall distribution of variability showed that loci differ in their contribution to the total observed diversity (H_T). The most variable loci were HVM 20 and HVM 60 (0.81) and the least variable was HVM 68(0.63). The proportion of diversity among and within populations indicated that, on average, 26% (G_{ST}) of the variation was among populations and 74% (1- G_{ST}) within populations (Table 7). The marker contributed differently to

Table 7. Partitioning of genetic differentiation (G_{ST}) of barley landraces within and between Korean, Chinese and Japanese populations, based on 7 polymorphic microsatellite loci

Locus	H_T	H_S	D_{ST}	G_{ST}
BMS64	0.71	0.41	0.31	0.43
HVKASI	0.72	0.48	0.25	0.34
HVM6	0.67	0.52	0.15	0.23
HVM20	0.81	0.67	0.14	0.17
HVM68	0.63	0.36	0.27	0.43
HVM60	0.81	0.21	0.60	0.03
HVM14	0.68	0.54	0.14	0.21
MEAN	0.72	0.53	0.18	0.26

Explanations: H_T – total gene diversity, H_S – mean gene diversity, D_{ST} – average gene diversity, GST – genetic differentiation

the observed degree of population differentiation, varying from a low differentiation (3%) for HVM60 to a high (43%) for BMS64 and HVM68.

4. Discussion and conclusions

Although automated sizing of alleles using fluorescent-labeled SSR primers and the AB-PEC prism 373A sequencer is an efficient way to assess allelic diversity in a large number of genotypes (Diwan & Cregan 1997), nonetheless, small peak-to-peak variation is required against the internal ROX-labeled size standard and standard accession (K013016). Variation in the allele size, calculated against the in-lane TAMRA-labeled size standard, was found among and within gels. This variation in allele size was similar in magnitude to variation found in other studies (Smith 1995; Fregeau & Fourney 1993), ± 0.2 and ± 0.5 bp, respectively. In order to accurately size alleles, K013016 was run as a standard for every locus and plate, and variation among plates was based on the variation in K013016 allele sizes from different plates.

The average alleles per locus ranged from 2 to 6 for dinucleotide and from 3 to 9 for trinucleotide repeats, and relative allele sizes were verified by comparison to radio-labeled allele data (Cregan *et al*. 1994). However, it was difficult to compare many genotypes because a stutter peak was associated with 1-2 bp repeat, also in this study; though many alleles were found in HVKASI, it was a mononucleotide repeat. In this case, a constant peak was recorded for the allele size, but most of primers produced unambiguous peak. Temperature is a critical factor related to the precision of the ABI Prism 3100 Genetic Analyzer, thus, this instrument utilizes an array of sixteen capillaries that are injected with samples and run simultaneously in the same temperature.

The allele frequency distributions of each primer among Korean, Chinese and Japanese barley landraces were similar. The number of alleles was related to the nucleotide motif and repeat number in microsatellites. Because the distribution of allele frequencies at most microsatellite loci was normal, the analysis was based on the repeat number of main allele for each locus. Dinucleotide repeats are reported to be most common and polymorphic in plants (Morgante & Olivieri 1993; Li *et al*. 2001).

Two studies on barley (Nevo *et al*. 1986; Turpeinen *et al*. 2001) have indicated that genetic diversity is not randomly distributed but is associated with certain ecogeographical factors, primarily, climatic (temperature and water availability) and edaphic. Recently, Turpeinen *et al*. (2001) showed connections between ecogeograpical variables such as water availability, temperature and geography and microsatellite band pattern. In this study, 21 accessions (72.4% of the group A) (Fig. 6), represented a spring type barley and 24 accessions – high-altitude barley. Hence, it is a strong evidence of correlation between character variables and latitude and growth character (winter vs. spring growth habit); separation is probably due to different eco-geographic conditions. The group C was composed of 86 accessions; in majority, they represented Korean germplasms (83 accessions), clustered in the group III (Fig. 5). 81 accessions represented naked barley. The group D mainly consisted of accessions from Japan. These observations suggest that this group may be useful to distinguish accessions based on their latitude, hull and sowing habit.

Partitioning of genetic variability by means of gene diversity statistics (Nei 1978) indicated that, on average, 70% of SSR diversity was distributed within barley landrace regional populations and 26 % between these populations. This is consistent with findings of other studies, indicating that considerable genetic diversity is partitioned within, rather than between, barley regional populations (Nevo *et al*. 1979, 1986; Turpeinen *et al*. 2001) By contrast, Dawson *et al*. (1993) reported converse findings, when the number of studied populations was higher. Diversity between populations was greater than within populations.

In conclusion, SSR markers may be used for diversity analyses in breeding and natural populations or genebank materials to exploit the genotyping data for crop improvement and as *ex situ* conservation strategies of plant genetic resources. This study also shows that SSR markers are powerful tools to examine functional diversity. A rich barley gene pool can be very useful for meeting current and future challenges in barley raising.

References

Anderson J. A., Churchill G. A., Autrique J. E., Sorrell M. E. & Tanksley S. D. 1993. Optimizing parental selection for genetic linkage maps. Genome 36: 181-186.

Becker J. & Heum M. 1995. Barley microsatellite: allele variation and mapping. Plant Mol. Biol. 27: 835-845.

Clark H. B. 1967. The origin and early history of the cultivated barleys. Agric. Hist. Rev. 15: 1-18.

Cregan P. B., Bhagwat A. A., Akkaya M. S. & Rongwen J. 1994. Microsatellite fingerprinting and mapping of soybean. Methods in Molec. Cell Biol. 5: 49-61.

Dawson I. K., Chalmers K. J., Waugh R. & Powell W. 1993. Detection and analysis of genetic variation in *Hordeum sponteneum* populatios from Israel using RAPD markers. Molec. Ecol. 2: 151-159.

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

Diwan N. & Cregan P. B. 1997. Automated sizing of fluorescentlabeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. Theor. Appl. Genetics 95: 723-733.

Fregeau C. J. & Fourney R. N. 1993. DNA typing with fluorescently tagged short tandem repeats: a sensitive and accurate approach to human identification. BioTechn. 15: 100-119.

Harlan J. R. 1979. On the origin of barley, *Hordeum vulgare* (Graminea-triticinae). In: N. W. Simmonds (ed.). Evolution of Crop Plants, pp. 93-98. Longman Group, London.

Huang X. Q., Borner A., Roder M. S. & Ganal M. W. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor. Appl. Genetics 105: 699-707.

IBGS 1996. The 7th International Barley Genetics Symposium. University of Saskatchewan, Saskatoon, Canada.

Keim P., Olson T. C. & Shoemarker R. C. 1992. Evaluation of soybean RFLP marker diversity in adapted germplasm. Theor. Appl. Genetics 85: 205-212.

Li J. L., Deng H., Lai D. B., Xu F., Chen J., Gao G., Recker R. R., & Deng H. W. 2001. Toward high- throughput genotyping: dynamic and automatic software for manipulating large-scale genotype data usig fluorescently labeled dinucleotide markers. Genome Res. 11: 1304-1314.

Lin J., Jonathan K. J. M.; James A. S., Hunter S. B., Margaret H. M., William K. G., Ude N. & Benjamin F. M. 1996. Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA mapping techniques. Plant Molec. Biol. Repor. 14: 156-169.

Morgante M. & Olivieri A. M. 1993. PCR-amplified microsatellites as markers in plant genetics. Plant J. 3: 175-182.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceed. National Acad. Sci. 70: 3321-3323.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Nevo E., Beiles A. & Zohary D. 1986. Genetic resources of wild barley in the Near East: structure, evoluation and application in breeding. Biol. J. Linnean Soc. 27: 355-380.

Nevo E., Zohary D., Brown A. D. & Haber M. 1979. Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum*, in Israel. Evolution 33: 815-833.

Pakniyat H., Powell W., Baird E., Handley L. L., Robinson D., Scrimgeour C. M., Hackett C. A., Forster B. P., Nevo E. & Caligari P. D. 1997. AFLP variation in wild barley (*Hordeum spontaneum* C. Koch) with reference to salt tolerance and associated ecogeography. Genome 40: 332-341.

Plucknett D. L., Smith N. H., Willams J. T. & Anishetty N. M. 1987. Genebanks and the world's food. Princeton University Press, Princeton, NJ, USA.

Poehlman J. M. 1985. Adaptation and Distribution. In: D. C. Rusmusson (ed.). Barley, Agronomy series, 26, pp. 1-17. American Society of Agronomy Inc. CSSA. SSSA.

Röder M. S., Korzun V., Wendehake K., Plachke J., Tixier M. H., Leroy P. & Ganal M. W. 1998. A microsatellite map of wheat. Genetics 149: 2007-2023.

Rohlf N. J. 1997. NTSYS-PC: numerical taxonomy and multivariate analysis system. v 2.1. Applied Biostatistics. Exeter Software, New York.

Saghai Maroof M. A., Biyashev R. M., Yang G. P., Zhang Q. & Allard R. W. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal location, and population dynamics. Proceed. National Acad. Sci. 91: 5466-5470.

Smith R. N. 1995. Ccurate size comparison of short tandem repeatalleles amplified by PCR. BioTechn. 18: 112-128.

Strelchenko P. P., Gumbareva N. K., Kovalyova O. N. & Graner A. 1996. Geographical and breeding trends within Eurasian cultivated barley germplasm revealed by molecular markers. In: S. H. Vaughan, D. A. Kazutoshi, O. Kazuto, S. Kaworu & M. Shoji (eds.). Plant Genetic Resources: Charcterization and Evaluation, pp. 115-132. Japan Tsukuba, Research Council Secretariat of MAFF and National Institute of Agrobiological Resources, Kannondai 2-1-2.

Turpeinen T., Tenhola T., Manninen O., Nevo E. & Nissilä E. 2001. Microsatellite diversity associated with ecology factors in *Hordeum spontaneum* populations in Israel. Molec. Ecol. 10: 1577-1591.

Yeh F. C., Yang R. C. & Boyle T. 1999. POPGENE, version 1.31. Microsoft windows based freeware for population genetic analysis. (http://www.ualberta.ca/~fyeh/fyeh).