Korean J. Crop Sci. 47(5): 379~383(2002)

Genetic Diversity of Barley Cultivars as Revealed by SSR Masker

Hong-Sik Kim*†, Kwang-Geun Park*, Seong-Bum Baek*, Sae-Jung Suh*, and Jung-Hyun Nam*

**National Crop Experiment Station, RDA, Suwon 441-100, Korea*

ABSTRACT : Allelic diversity of 44 microsatellite marker loci originated from the coding regions of specific genes or the non-coding regions of barley genome was analyzed for 19 barley genotypes. Multi-allelic variation was observed at the most of marker loci except for HVM13, HVM15, HVM22, and HVM64. The number of different alleles ranged from 2 to 12 with a mean of 4.0 alleles per microsatellite. Twenty-one alleles derived from 10 marker loci are specific for certain genotypes. The level of polymorphism (Polymorphic Information Content, PIC) based on the band pattern frequencies among genotypes was relatively high at the several loci such as HVM3, HVM5, HVM14, HVM36, HVM62 and HVM67. In the cluster analysis using genetic similarity matrix calculated from microsatellite-derived DNA profiles, two major groups were classified and the spike-row type was a major factor for clustering. Correlation between genetic similarity matrices based on microsatellite markers and pedigree data was highly significant (r=0.57), but these two parameters were moderately associated each other. On the other hand, RAPD-based genetic similarity matrix was more highly associated with microsatellite-based genetic similarity (r=0.63**) than coefficient of parentage.**

Keywords **:** barley, microsatellite, DNA marker, genetic variation, coefficient of parentage, RAPD, genetic similarity

When DNA sequences are determined in both plant and animal genomes, tandom repeats of one or up to 7 nucleotide sequence motifs are observed. These simple sequence repeats (SSR), also called microsatellites are flanked with unique sequences. The polymorphism of microsatellites is derived from the length variability of repeated sequences and can be detected by PCR (polymerase chain reaction) using these flanked unique sequences as forward/reverse primers.

The high variability of microsatellites are reported in plant species including rice, maize, soybean, barley and wheat (Wu & Tanksley, 1993; Senior & Heun, 1993; Akkaya *et al.*, 1992; Saghai Maroof *et al.*, 1994; Röder *et al.*, 1998). It has been known from the previous studies that microsatellites are abundant in the genome and are not limited to specific chromosomes but distributed throughout the entire genome. With this regard, microsatellites are important as typical DNA markers for genetic mapping for qualitative and quantitative traits and even for classification of genotypes in crop species.

In barley, Saghai Maroof *et al.* (1994) first evaluated the extent of genetic variation of microsatellite regions. As many as 28 and 37 alleles were observed at two microsatellite loci, respectively in a sample of 207 accessions of wild and cultivated barley. In their studies, microsatellites were used as markers to trace the changes of genetic make-up in the population. Becker and Heun (1995) reported the development of microsatellites. Most of microsatellites originated from the coding sequences of specific genes through a database search from GenBank and EMBL (Becker and Heun, 1995). Liu et al. (1996) also identified a total of 55 microsatellites after screening 36,000 recombinant clones of a barley genomic library with $(GA)_{10}$ and $(CA)_{10}$ probes and finally added 45 microsatellite markers to the frame map of RFLP and other markers. They demonstrated the possibility of microsatellites as markers in genetic mapping studies in barley.

In our studies, the data on genetic polymorphism of microsatellite DNA markers were generated for a set of barley genotypes. Variation at each of microsatellite DNA marker loci was evaluated and compared with other types of DNA marker and parentages of genotypes as measures of genetic diversity. The polymorphic marker data will be used for further studies of genetic mapping.

MATERIALS AND METHODS

Plant materials

A set of 19 barley genotypes were measured for microsatellite polymorphisms. Fourteen genotypes were domestically developed varieties and lines. These are six-rowed covered barley cultivars such as 'Olbori', 'Daebaekbori', 'Chalbori', 'Seodunchalbori', 'Milyang71', 'Chogangbori', and 'Namhaebori', six-rowed naked barley cultivars such as 'Baekdong', 'Neulssalbori', and 'Chalssalbori', and two-rowed covered barley cultivars such as 'Jinyangbori', 'Sacheon6', 'Doosan6', and 'Suwon346'. Five genotypes

†Corresponding author: (Phone) +82-31-290-6677 (E-mail) kimhongs@rda.go.kr <Received November 21, 2002>

are foreign introduced cultivars such as 'Karl' (6-rowed and covered), 'Mokusekko3' (6-rowed and covered), 'Franka' (6-rowed and covered), 'Yonezawa Mozi' (6-rowed and naked), and 'Ishuku Shirazu' (2-rowed and covered). Some of these materials have favorable genes for disease resistance and good quality for end-use, and were used as parents for population development.

PCR of microsatellites

A total of 44 primer pairs flanking the specific microsatellite sites were used in the PCR amplification. These primers were developed by Saghai Maroof and his group (Saghai Maroof, *et al.*, 1994; Liu, *et al.*, 1996) and currently commercially available (http://www.resgen.com/). The names of individual microsatellite loci analyzed in this study and their chromosomal locations are listed in Table 1.

The 10 μl PCR mixture consisted of 200 μM of each dNTPs, 40 ng of each primer, 50 ng of template DNA, 1 unit *Taq* DNA polymerase (Promega, Madison, USA), 10 mM Tris-HCl, 50 mM KCl and 1.5 mM $MgCl_2$. The PCR amplifications were done with thin-walled 0.5 ml tubes in an PTC-200 (MJ Research Inc, Massachusetts, USA). One of the five different PCR conditions was suggested for individual primer pairs (Liu *et al.*, 1996; Becker and Heun, 1995; Röder *et al.*, 1995) and the reactions were performed by using these conditions in this study.

The PCR product was mixed with 3 μl of stopping buffer (70% glycerol, 20 mM EDTA, 0.2% SDS, 0.6 mg/ml bromophenol blue) and 4-5 μl of reaction mixture was used for 5% polyacrylamide gel electrophoresis. The amplified DNA bands were visualized by the silver staining method (Promega, Madison, USA).

Data collection and Analysis

Silver-stained DNA bands were visually scored as present or absent. The number and frequency of band patterns per primer pairs were determined and used for the calculation of the Polymorphic Information Content (PIC) index (Anderson *et al.*, 1993). Genetic similarity based on the polymorphic bands between two genotypes was calculated using Nei and Li's computation (1979). The SAHN cluster routine (Rohlf, 1992) using the UPGMA (unweighted pair group method, arithmetic average) option was used to generate dendrogram based on the microsatellite-based genetic similarity estimates.

The data for RAPD marker polymorphism among 18 genotypes except for 'Milyang71' were obtained from the results of previous study (Kim *et al.*, 2000). Data collection and analysis for RAPD were same as those for microsatellites. RAPD-based genetic similarity estimates were compared with microsatellite-based results to determine the relationship between the different DNA marker-based measures of genetic diversity.

A subset of 13 barley cultivars was included in the pedi-

Table 1. Allelic variation of 31 microsatellite DNA marker loci in barley.

SSR locus	Repeat†	Chr‡	No.of alleles	PIC	SSR locus	Repeat	Chr.	No.of alleles	PIC
HVCMA	$(AT)_9$	1	4	0.523	HVM67	$(GA)_{11}$	4	5	0.784
HVM4	$(AT)_9$	1	4	0.648	HVM68	$(GA)_{22}$	4	4	0.628
HVM5	$(GT)_6$, $(AT)_{16}$	1	12	0.771	HVM20	$(GA)_{19}$	5	4	0.689
HVM51	$(GA)_3$, $(GGGA)_3$, $(GA)_8$	1	2	0.269	HVM43	$(CA)_9$	5	6	0.670
HVBKASI	$(C)_{10}$, $(A)_{11}$	2	2	0.466	HVM64	$(GA)_4$, $(GT)_7$, $(CT)_2$, $(GT)_4$, $(GA)_8$	5	1	0.000
HVCSG	$(CA)_4$, $(C)_{17}$	2	5	0.723	HVM14	$(CA)_{11}$	6	3	0.809
HVM36	$(GA)_{13}$	2	6	0.764	HVM22	$(AC)_{13}$	6	1	0.000
HVM54	$(GA)_{14}$	2	7	0.595	HVM31	$(AC)_9$	6	3	0.658
HVM9	$(TCT)_5$	3	2	0.394	HVM65	$(GA)_{10}$	6	2	0.553
HVM15	$(GA)_8$	3	1	0.000	HVM74	$(GA)_{13}$	6	6	0.744
HVM27	$(GA)_{14}$	3	6	0.466	HVDHN9	$(AC)_6$	7	4	0.435
HVM44	$(GA)_8$	3	2	0.269	HVLEU	$(ATTT)_4$	7	2	0.332
HVM62	$(GA)_{11}$	3	10	0.851	HVM6	$(GA)_9$	7	3	0.591
HVM3	$(AT)_{29}$	4	8	0.824	HVM7	$(AT)_7$	7	2	0.487
HVM13	$(GA)_6$, $(GA)_6$, $(GA)_6$	4	1	0.000	HVM30	$(CA)_8$	7	2	0.435
HVM40	$(GA)_6$, $(GT)_4$, $(GA)_7$	4	5	0.742					

†‡The data for each marker locus are from Lie *et al.* (1996)

gree analysis to determine correlation between microsatellite-based genetic similarity and coefficients of parentage. Six genotypes including 'Baekdong', 'Suwon 346', 'Karl', 'Mokusekko3', 'Franka', and 'Ishuku Shirazu' were excluded. Pedigrees of individual genotypes were obtained from several data sources: release notices, plant breeder's documents of barley genealogy, GrainGene internet database (http://www.wheat.pw.usda.gov), and barley germplasm internet database in the Okayama University, Japan (http://www.rib.okayama-u.ac.jp/barley/). Coefficients of parentage for genotype pairs were computed as following Kempthorne (1969) and Cox *et al.* (1986).

RESULTS AND DISCUSSION

We analyzed allelic diversity of microsatellite marker loci originated from either the coding regions of specific genes or the non-coding regions of barley genome. Thirty-one of the 44 microsatellite primer pairs gave clear band patterns in the expected size ranges. Eleven primer pairs generated smear bands and/or no PCR products.

The number of variants (*i.e.*, alleles) varied widely at the 31 loci (Table 1). Multi-allelic variation was observed except for four primer pairs including HVM13, HVM15, HVM22, and HVM64. The number of different alleles ranged from 2 to 12 with a mean of 4.0 alleles per microsatellite. In particular, 12 different alleles were segregated at the HVM5 locus of chromosome 1. This means that there are 12 DNA sequence repeats with different length in the 19 genotypes for HVM5 locus (Saghai Maroof *et al.*, 1994). Examples of multi-allelic variation of 19 barley genotypes at HVM36 and HVM40 are shown in Fig. 1. Twenty-one alleles derived from 10 marker loci are specific for certain genotypes. These unique alleles are quite often found especially in 'Franka', 'Karl' and 'Mokusekko3'. Some of these unique markers may be important for identification of genotypes among germplasms.

The level of polymorphism at each locus was calculated by using the polymorphic information content (PIC) based on the band pattern frequencies among genotypes. The PIC values indicate how much the population is heterogeneous for a specific locus. Relatively high PIC values (>0.75) were observed at some loci such as HVM3, HVM5, HVM14, HVM36, HVM62, and HVM67 (Table 1). In particular, PIC value of HVM14 at which only three alleles were found was 0.809. This indicates that combination of these alleles is not biased to a certain genotype at this locus.

When screened 207 accessions of cultivated and wild barleys for microsatellite polymorphism, up to 37 alleles have been reported at locus HVM4 (Saghai Maroof *et al.* 1994). With this regard, Saghai Maroof *et al.* (1994) have already suggested great potential of microsatellite markers for the genetic mapping in barley since they proved high level of allelic diversity. Microsatellite polymorphism was surveyed among the parental lines of the 4 mapping population and its result is shown in Table 2. The level of genetic polymor-

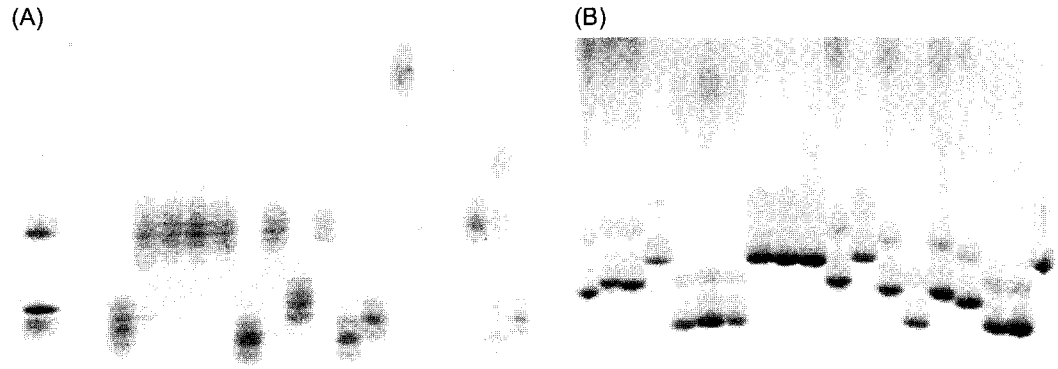

Fig. 1. Multi-allelic variation of 19 barley cultivars at the microsatellite loci such as (A) HVM36 and (B) HVM40.

Table 2. Frequencies of microsatellite polymorphism in the parents of population.

Parents for populations	Trait	No. of microsatellites tested	No. of polymorphic microsatellites (%)
Yonezawa Mozi × Neulssalbori	β-glucan content	37	8 (21.6)
Chalbori × Baekdong	BaYMV resistance	37	16 (43.2)
Sacheon 6 × Namhaebori	BaYMV resistance	37	24 (64.9)
Karl × Jinyangbori	Seed protein content	37	18 (48.6)

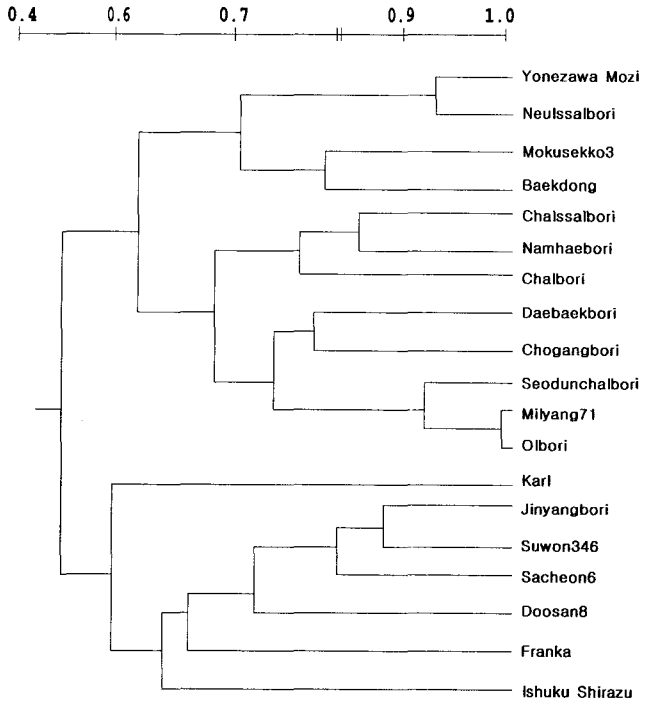

Fig. 2. Cluster analysis of 19 barley cultivars based on Nei & Li's genetic similarity(GS) coefficient of microsatellite markers.

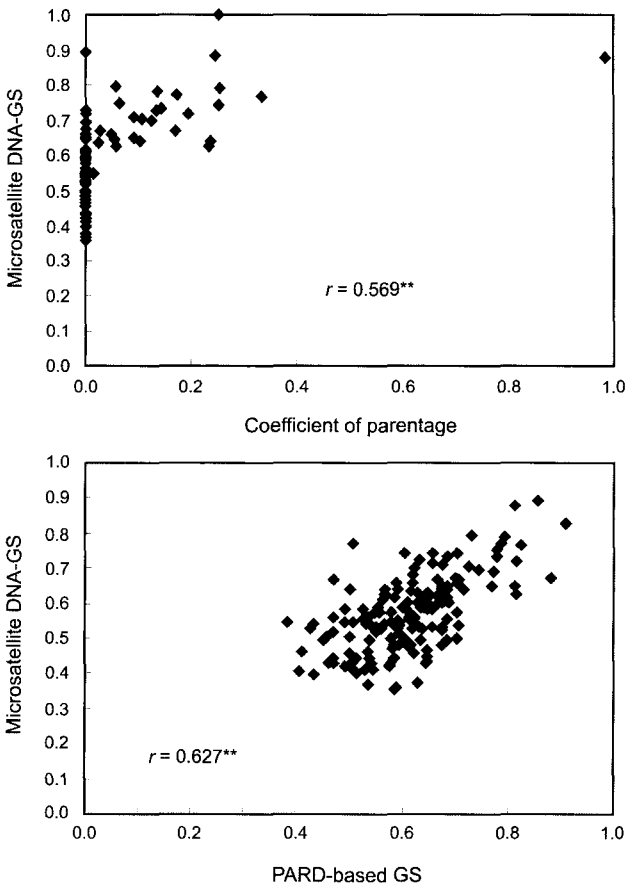

Fig. 3. Correlations of microsatellite DNA marker-based genetic similarity(GS) with COP(above) and RAPD-based GS(below), respectively.

phism is various and dependent upon genotype pairs. Only 8 microsatellites were polymorphic between two hulless barley cultivars, 'Yonezawa Mozi' and 'Neulssalbori'. On the other hand, more than 50% of microsatellites assayed in this study were polymorphic between 2-rowed and 6-rowed barley cultivars, 'Sacheon 6' and 'Namhaebori'. Since these polymorphic markers are already mapped over the 7 chromosomes, they will be used as anker markers to assign linkage groups to the respective chromosomes in the genetic mapping studies. These results suggest that microsatellite is a quite invaluable marker type having advantages of high probability of polymorphism and genetic information for genetic linkage analysis in barley.

In order to evaluate microsatellite DNA markers to access genetic relationships among genotypes, microsatellite-derived DNA profiles were used for clustering of individuals. Similarity values were calculated based on 125 microsatellite bands, and a dendrogram was constructed based on the similarity matrix (Fig. 2). Two major groups were classified and the spike-row type was a major factor for clustering. As shown in Fig. 2, all five accessions of two-rowed barley cultivars including 'Jinyangbori', 'Suwon346', 'Sacheon 6', 'Doosan 8' and 'Ishuku Shirazu' were grouped in a subcluster. The other six-rowed covered and naked barley cultivars except for 'Karl' and 'Franka' belonged to the other subcluster. This result indicates that the two-rowed barley pool is quite different taxonomically from the six-rowed barley pool even if the spike-row type only is a major trait to distinguish these two pools. Genomic differentiation of barley cultivars between these two pools is greater than that within a pool in terms of polymorphism in the microsatellite region.

The association of microsatellite-based genetic relationships among genotypes with other parameters for genetic diversity was determined as shown in Fig. 3. Correlation between genetic similarity matrices based on microsatellite markers and pedigree data was highly significant (r=0.57), but these two parameters were moderately associated each other. Coefficient of parentage between two genotypes indicates the genetic proportion shared by two genotypes identical by descent. On the other hand, microsatellite-based genetic similarity estimates include the genetic proportion of two genotypes identical in state as well as the proportion of genetic relationship by common parentages (Kempthorne, 1969). As compared to coefficient of parentage, RAPD-based genetic similarity matrix was relatively highly associ-

ated with microsatellite-based genetic similarity (Fig. 3). This is because genetic proportion identical by descent and also in state was measured by both DNA markers.

Some microsatellites analyzed in this study originated from the coding sequences of specific genes such as those coding for β-ketoacyl-acyl carrier protein synthasel isoenzyme (HVBKASI), thiol protease aleurin (HVLEU), dehydin-9 (HVDHN9) and β-amylase inhibitor (HVCMA) (Becker & Heun, 1995). However, microsatellites are usually located within non-coding DNA sequences and are prone to slippage during DNA replication. Changes of any length of repeat sequence due to either insertion or deletion of repeating units appear genetic instability of markers. Therefore, DNA fingerprints derived from microsatellite DNA only may not be ideally suited for the identification and registration of genotypes.

The present study shows that microsatellite-derived DNA fingerprints are reliable as measures of genetic diversity and genetic relationships even though they do not accurately access the genetic relationships of cultivars in some cases. With multi-allelic variation at individual marker loci, they also can be very useful as genetic markers in tracing target genes for introgression in the backcross breeding program.

It is expected that more microsatellite DNA markers will be developed as the internationally collaborative efforts such as Triticeae genome mapping project (http://wheat.pw.usda.gov/genome/index.html) are made for the development of ESTs and database of their sequence information. In barley, specific primer sequences for over 600 microsatellite loci are known to be available. Due to the advantages of their abundance in genome, an easy technique of marker genotyping, and potential informativeness of genetics, microsatellite DNA will be extensively exploited in the basic genomic studies and in the applied molecular breeding system.

REFERENCES

Akkaya, M. S., A. A. Bhagwat, and P. B. Cregan. 1992. Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics* 132 : 1331-1339.

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

Becker, J. and M. Heun. 1995. Barley microsatellites: allele variation and mapping. *Plant Mol. Biol.* 27: 835-845.

Cox, T. S., J. P. Murphy, and D. M. Rodgers. 1986. Changes in genetic diversity in the red winter wheat regions of the United States. *Proc. Natl. Acad. Sci. USA* 83: 5583-5586.

Kempthorne, O. 1969. An introduction to genetic statistics. Iowa State University Press, Ames, Iowa.

Kim, H. S., K. G. Park, J. C. Kim, J. G. Kim, M. W. Park, and J. H. Nam. 2000. Genetic diversity of Korean barley cultivars. Proceedings of Workshop for Seed War and Biotechnology in the 21st Century, Korean Soc. of Breeding. pp127-128.

Liu, Z.-W., R. M. Biyashev, and M. A. Saghai Maroof. 1996. Development of simple sequence repeat DNA markers and their integration into a barley inkage map. *Theor. Appl. Genet.* 93 : 869-876.

Nei, M. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76 : 5269-5273.

Röder, M. S., J. Plaschke, S. U. Konig, A. Borner, M. E. Sorrells, S. D. Tanksley, and M. W. Ganal. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. Gen. Genet.* 246 : 327-333.

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

Rohlf, F. J. 1992. NTSYS-pc, Numerical taxonomy and multivariate analysis system. ver. 1.7. Exter Publ., Setauket. New York, USA

Saghai Maroof, M. A., R. B. Biyashev, G. P. Yang, Q. Zhang, and R.W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. *Proc. Natl. Acad. Sci. USA* 91 : 5466-5470.

Senior, M. L. and M. Heun. 1993. Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. *Genome* 36 : 884-889.

Wu, K.-S. and S. Tanksley. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. *Mol. Gen. Genet.* 241 : 225-235.