

Genetic Diversity Measured by RAPDs in Korean Barley Germplasm Pools

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ABSTRACT : Molecular-based genetic diversity for a set of 141 accessions of Korean barley cultivars and 24 accessions of foreign exotic cultivars were analyzed using random amplified polymorphic DNAs (RAPDs). Different level of genetic variability was observed with 30 random decamer primers in the Korean barley varieties and breeding lines which were preliminarily classified by morphological (hulled & hulless barley) and end-use (malting barley) and/or by the released periods. A total of 74 RAPD bands were scored, and the number of bands per primer varied from 1 to 7 with an average of 2.74. The hulled barley pool had one more marker genotype per primer than the hulless barley pool. The polymorphic information content (PIC) values based on the band pattern frequencies among genotypes varied depending on genetic pools where mean PICs of hulled, hulless and malting barleys were 0.62, 0.57, and 0.43, respectively. Certain genomic loci amplified by opR04, opF01, opB05, and opC13 were highly polymorphic with PIC > 0.8. Patterns and temporal trends of genetic diversity assessed over the period from 1970s to 1990s had a tendency to increase, and in particular, this upward slant was quite clear and significant for the hulless barley pool. In the cluster analysis using genetic similarity matrix calculated from RAPD profiles, two major groups and several small subgroups were classified. Major grouping of materials was not affected by the presence of the husk but by their genetic background and the spike-row type. The validity of information on the genetic diversity and relationships between genotypes will have been reviewed to predict their yield potential.

Keywords: barley, genetic diversity, RAPD, hulled, hulless, malt, germplasm

Barley is one of the major food crops and recently its value as a feed crop has also been on the rise in Korea. Systematic and scientific breeding for Korean barley cultivars began in the late 1950s since the first selection of elite lines among the collected landraces in 1908. Most efforts were made on the improvement of some agronomic traits such as high yield, early maturity, winter-hardiness and lodging resistance in the early days of barley breeding.

Breeding objectives have been changed and varied widely in accordance with the improvement of different end-use qualities since 1980s.

As reviewed in the previous reports (NCES, 1998; WBRI, 1987), great achievements were observed in the relative short period for improvement of the most targeted traits such as yield and early maturity in barley. This is based upon recombination and selection of favorable genes from the limited source of the adapted and elite lines or exotic germplasm (Jong & Park, 2000a, b). However, such a progress in the long term goal for the development of new barley cultivars will be primarily dependent upon the amount and quality of genetic variation existed among the breeding materials. Plant breeders could understand indirectly how much their breeding materials were diverged with development of statistical and biotechnological tools. Use of morphological traits observed in the field can be one of the approaches to measure the apparent genetic diversity in the population. Baek *et al* (2000) reviewed morphological variation of a total of 145 accessions of Korean barley cultivars in terms of their cultivation periods for 10 quantitative and 31 qualitative traits. Jong & Park (2000a, b) measured genetic diversity of hulled and hulless Korean barley cultivar pools by using coefficient of parentage analyses.

With the application of DNA markers as a molecular tool, more accurate estimation of genetic diversity for barley germplasm pools could be possible. Peterson *et al* (1994) and Graner *et al* (1994) accessed genetic variation over a few number of RFLP marker loci for the wild (*Hordeum spontaneum*) and/or cultivated barley (*H. vulgare*) pools. Dahleen (1997) identified a few informative RFLP marker loci at which high number and frequency of banding patterns were detected among 28 North American barley cultivars. Russell *et al* (1997) evaluated and compared similarity measures based on four different DNA marker systems on a set of cultivated European barley germplasm.

As for the Korean barley germplasms, only part of some Korean barley varieties were evaluated when DNA fingerprinting using RAPD and STS assays was determined (Jang *et al*, 2001) or genetic variation of SSR marker loci was measured (Kim *et al*, 2002). So far, extensive studies of molecular-based genetic diversity for a set of Korean barley

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cultivars has not been reported yet. In our studies, genetic diversity was analyzed using RAPDs for Korean barley cultivars and breeding lines which were primarily classified by morphological (covered & naked barley pools) and end-use (malting barley pools) and/or by the released periods. This information may act as a guideline for the use and reassem-

bling of exiting genetic diversity of barley genetic resources in the breeding programs.

MATERIALS AND METHODS

A total of 165 barley cultivars including 78 cultivars of

Table 1. List of 165 accessions of hulled, hulless, malt, and exotic barley germplasms.

Name	Row	Year	Origin	Group	Name	Row	Year	Origin	Group
HULLED									
Samheung	6			II-a	Suwon216	6	1999	Suwon	I-a
Suwon18	6	1932	Suwon	I-d	Suwon229	6	1999	Suwon	I-a
Buheung	6	1963	Suwon	I-d	Suwon231	6	1999	Suwon	I-a
Yeogi	6	1967	Suwon	I-d	Suwon232	6	1999	Suwon	I-a
Hangmi	6	1967	Suwon	I-d	Suwon233	6	1999	Suwon	I-a
Olbori	6	1973	Suwon	II-a	Suwon238	6	1999	Suwon	I-a
Kangbori	6	1976	Suwon	I-a	Suwon239	6	1999	Suwon	I-a
Bunong	6	1976	Suwon	I-d	Suwon249	6	1999	Suwon	I-g
Dongbori1	6	1977	Suwon	I-f	Suwon251	6	1999	Suwon	II-a
Dongbori2	6	1977	Suwon	I-f	Suwon259	6	1999	Suwon	I-c
Durubori	6	1978	Suwon	I-g	Suwon260	6	1999	Suwon	I-d
Jogangbori	6	1980	Suwon	II-a	Suwon290	6	1999	Suwon	I-a
Tapgolbori	6	1981	Suwon	II-a	Suwon299	6	1999	Suwon	I-*
Paldalbori	6	1982	Suwon	I-*	Suwon300	6	1999	Suwon	II-a
Saeolbori	6	1983	Suwon	I-a	Suwon307	6	1999	Suwon	II-a
Chalbori	6	1984	Suwon	I-a	Suwon308	6	1999	Suwon	I-c
Saekangbori	6	1993	Suwon	I-g	Suwon309	6	1999	Suwon	II-a
Seodunchalbori	6	1996	Suwon	II-a	Suwon310	6	1999	Suwon	II-a
Owolbori	6	1979	Milyang	I-f	Suwon311	6	1999	Suwon	I-c
Namhaebori	6	1981	Milyang	I-a	Suwon318	6	1999	Suwon	II-a
Alchanbori	6	1983	Milyang	I-*	Suwon319	6	1999	Suwon	I-a
Daejinbori	6	1989	Milyang	I-*	Suwon320	6	1999	Suwon	I-a
Milyangketbori	6	1992	Milyang	I-*	Suwon328	6	1999	Suwon	I-a
Saealbori	6	1992	Milyang	I-g	Suwon330	6	1999	Suwon	I-a
Keunalbori	6	1993	Milyang	I-g	Suwon331	6	1999	Suwon	I-*
Nagyeongbori	6	1994	Milyang	I-a	Suwon338	6	1999	Suwon	I-a
Daebekbori	6	1994	Milyang	I-*	Suwon339	6	1999	Suwon	I-a
Miragbori	6	1996	Milyang	I-g	Suwon340	6	1999	Suwon	I-a
Suwon200	6	1999	Suwon	II-a	Suwon347	6	1999	Suwon	I-a
Suwon201	6	1999	Suwon	I-g	Suwon348	6	1999	Suwon	I-a
Suwon202	6	1999	Suwon	I-g	Suwon349	6	1999	Suwon	I-a
Suwon203	6	1999	Suwon	I-g	Suwon354	6	1999	Suwon	I-a
Suwon204	6	1999	Suwon	I-*	Suwon355	6	1999	Suwon	I-c
Suwon205	6	1999	Suwon	I-f	Suwon359	6	1999	Suwon	I-b
Suwon206	6	1999	Suwon	II-a	Suwon361	6	1999	Suwon	I-b
Suwon207	6	1999	Suwon	I-b	Suwon362	6	1999	Suwon	I-g
Suwon208	6	1999	Suwon	I-a	Suwon363	6	1999	Suwon	I-g
Suwon209	6	1999	Suwon	I-c	Suwon365	6	1999	Suwon	II-a
Suwon214	6	1999	Suwon	I-e	Suwon366	6	1999	Suwon	I-a

Table 1. Cont'd.

Name	Row	Year	Origin	Group	Name	Row	Year	Origin	Group
<u>HULLESS</u>									
Masankwamaek(P)	6			I-e	Suwon243	6	1999	Suwon	I-b
Masankwamaek(Y)	6			I-e	Suwon253	6	1999	Suwon	II-a
Baekdong	6	1936	Iksan	I-e	Suwon254	6	1999	Suwon	I-a
Cheongmaek	6	1942	Jinju	I-e	Suwon261	6	1999	Suwon	I-a
Guigwa	6	1948	Daegu	I-e	Suwon262	6	1999	Suwon	II-a
Youngsanbori	6	1966	Naju	I-e	Suwon271	6	1999	Suwon	II-a
Gwangsung	6	1973	Naju	I-b	Suwon286	6	1999	Suwon	II-a
Mokpo51	6	1979	Suwon	I-e	Suwon291	6	1999	Suwon	I-c
Songhagbori	6	1982	Iksan	I-e	Suwon293	6	1999	Suwon	I-c
Saessalbori	6	1983	Iksan	I-e	Suwon294	6	1999	Suwon	I-c
Nulssalbori	6	1984	Iksan	I-e	Suwon302	6	1999	Suwon	I-c
Moodeungssalbori	6	1986	Naju	I-e	Suwon303	6	1999	Suwon	I-b
Chalssalbori	6	1988	Suwon	I-b	Suwon312	6	1999	Suwon	I-c
Kinssalbori	6	1988	Iksan	I-e	Suwon313	6	1999	Suwon	I-c
Nachanssalbori	6	1988	Iksan	I-b	Suwon321	6	1999	Suwon	I-a
Hinssalbori	6	1992	Iksan	I-e	Suwon322	6	1999	Suwon	I-b
Hinchalssalbori	6	1993	Iksan	I-e	Suwon323	6	1999	Suwon	I-b
Saechalssalbori	6	1994	Suwon	I-c	Suwon324	6	1999	Suwon	I-b
Olssalbori	6	1994	Iksan	I-e	Suwon333	6	1999	Suwon	I-a
Chunchussalbori	6	1995	Iksan	I-e	Suwon341	6	1999	Suwon	I-*
Duwonchapssalbori	2	1996	Suwon	II-c	Suwon342	6	1999	Suwon	I-b
Kwanghwalsalbori	6	1996	Iksan	I-e	Suwon343	6	1999	Suwon	II-a
Ganghossalbori	6	1997	Iksan	I-e	Suwon350	6	1999	Suwon	I-e
Jinmichapssalbori	6	1998	Suwon	I-a	Suwon351	2	1999	Suwon	II-c
Daehossalbori	6	1998	Iksan	I-a	Suwon356	6	1999	Suwon	I-b
Suwon241	6	1999	Suwon	I-b	Suwon357	6	1999	Suwon	I-e
Suwon242	6	1999	Suwon	I-b					
<u>MALT</u>									
Sacheon 6	2	1979		II-c	Jinyangbori	2	1993	Suwon	II-c
Dusan 8	2	1981		II-c	Samdobori	2	1993	Milyang	II-c
Dusan 29	2	1988		II-c	Namhyangbori	2	1995	Suwon	II-c
Jinkwangbori	2	1989	Suwon	II-c	Suwon212	2	1999	Suwon	I-b
Jejubori	2	1992	Suwon	II-c	Suwon346	2	1999	Suwon	II-c
<u>EXOTIC</u>									
Atahualpa	2	Hulless	Ecuador	II-a	Chikurin Ibaraki 1	6	Hulled	Japan	I-d
Diana	2	Hulled	Germany	II-b	Dixcen-Hiproly	6	Hulled		II-a
Gobernadora	2	Hulled	Mexico	II-c	Franka	6	Hulled	Germany	II-b
Igrı	2	Hulled	Germany	II-b	Frederickson	6	Hulled	Sweden	II-c
Isabell	2	Hulled	Germany	II-b	Haganemugi	6	Hulled	Japan	I-a
Ishukushirazu	2	Hulled	Japan	II-c	Karl	6	Hulled	USA	II-b
Mikamo Golden	2	Hulled	Japan	II-c	Mihorihadaka3	6	Hulled	Japan	I-d
Misato Golden	2	Hulled	Japan	II-c	MNBrite	6	Hulled	USA	II-b
Sonate	2	Hulled	Germany	II-b	Mokusekko3	6	Hulled	Japan	I-d
Svanhals	2	Hulled	Germany	II-c	Nakadehadaka	6	Hulled	Japan	I-e
Zhedar	2	Hulled	China	II-c	Kikaihadaka	6	Hulless	Japan	I-e
Chevron	6	Hulled	Switzerland	II-b	Yonezawa Mochi	6	Hulless	Japan	I-e

covered barley, 53 of naked barley, 10 of malting barley and 24 cultivars of foreign germplasm were used for RAPD analysis (Table 1). Suwon-bred lines were presumed to be developed in 1999 in the study.

Barley DNA was extracted from the greenhouse-grown plants by a modified CTAB procedure (Saghai Maroof *et al.*, 1984). Pulverized leaf tissues were incubated for one hour at 63–65 °C with CTAB extraction buffer (2% CTAB, 1.4M NaCl, 0.2M EDTA, 0.1M Tris-HCl pH 8.0, 1.0% 2-mercaptoethanol). The aqueous phase was then extracted twice with chloroform:isoamyl alcohol [24:1(v/v)] and the total genomic DNA was precipitated with ice-cold 95% ethanol. DNA was resuspended with TE buffer and RNA was removed with RNase.

For RAPD analysis, 20 ng of genomic DNA of each cultivar, 0.3 µM primer, 1 reaction buffer (10 mM Tris-HCl, pH 8.3, 10 mM KCl), 2.5 mM MgCl₂, 0.5 mM each dNTP, and 1 unit of Taq polymerase (Promega, Madison, WI). DNA amplification was done using a thermal cycle profile of 5 min at 93 °C followed by 45 cycles of 1 min at 93 °C, 1 min at 36 °C, 2 min at 72 °C, and additional 10 min at 72 °C. Random decamer primers obtained from Operon Technologies Inc., Alameda, CA. were used for polymerase chain reaction (PCR). DNA amplification was done by using PTC-200™ Programmable Thermal Controller (MJ Research Inc., Watertown, MA). PCR products were separated on a 1.4% (w/v) agarose horizontal gel and visualized by ethidium bromide staining.

Amplified DNA bands on the gel were visually scored as present or absent. The number and frequency of band patterns per primer were determined and used for calculation of the Polymorphic Information Content (PIC) index (Anderson *et al.*, 1992):

$$PIC_i = 1 - \sum P_{ij}^2$$

where P_{ij} is the frequency among the assayed genotypes of the j th pattern of primer i .

Genetic similarity (GS) based on RAPD markers between two genotypes, x and y , was estimated using simple matching coefficient computation. Genetic relationships among genotypes were determined by the SAHN cluster routine (Rohlf, 1992) using UPGMA (unweighted pair group method, arithmetic average) based on the RAPD-based GS estimates.

RESULTS AND DISCUSSION

In the analysis of genetic variability for a set of 165 barley accessions including 24 foreign cultivars, a total of 74 RAPD bands with different size was observed with 30 primers. A typical polymorphic banding pattern of RAPD is shown in Fig. 1. The number of bands produced by each primer varied from one for opE07, opF16, opN11, opU01 and opW16 to as many as seven for opR04, with an average of 2.74. These primers were selected in preliminary experiments for the reproducible DNA bands generated using barley templates.

The hulled and hulless barley pools had the same number of polymorphic bands of 73 even if more accessions of the hulled barley pool were analyzed (Table 2). However, the hulled barley pool showed on the average one more band pattern per primer than the hulless barley pool. When the released Korean varieties were examined, 29 out of 69 polymorphic bands for hulled barley, 31 out of 61 polymorphic bands for hulless barley and 19 out of 43 polymorphic bands for two-rowed malting barley were observed in frequencies of ≥ 0.5 . Almost all bands were shared by the Korean barley pool and exotic germplasm pool except for one band of 'Nakadehadaka' from Japan.

Polymorphic information content (PIC) value estimated in each pool was consistent with the other parameters of genetic diversity such as numbers of polymorphic bands and of band patterns. The mean PIC was 0.65 over all accessions, and varied depending on germplasm pools and

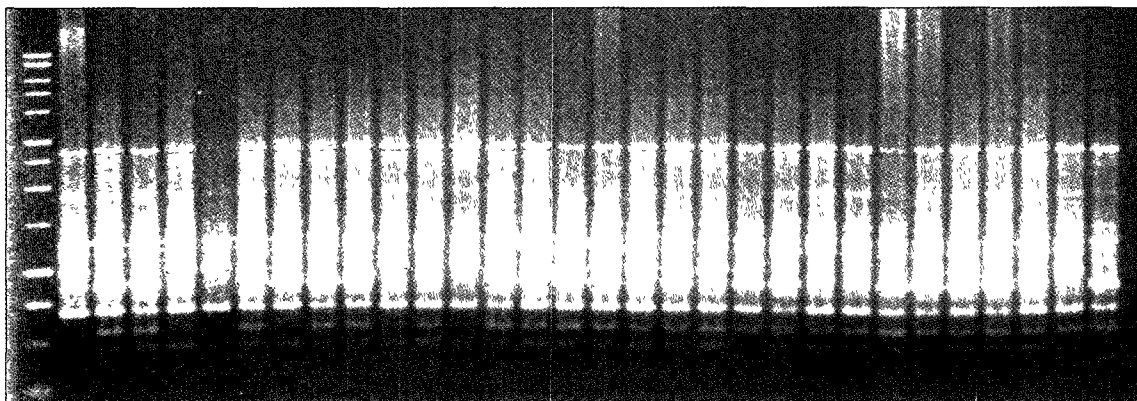


Fig. 1. Polymorphic DNA band profiles of Suwon-breeding lines of barley as revealed by opV14 primer.

Table 2. RAPD-based genetic variability among different barley germplasm pools.

	Total	Hulled barley (Korea)	Hulless barley (Korea)	Malting barley (Korea)	Foreign germplasm
No. of accessions	165	78	53	10	24
No. of polymorphic bands	74	73	73	58	74
No. of banding patterns per primer [†]	7.93	6.56	5.56	2.78	4.93
Avg. PIC [‡]	0.65	0.62	0.57	0.43	0.64
Avg. GS [§]	0.628	0.644	0.676	0.785	0.612
Max. GS	0.986	0.986	0.972	0.918	0.946
	(Suwon309- Suwon300, Suwon307)	(Suwon309- Suwon300, Suwon307)	(Mudeungssalbori- Saessalbori)	(Jinyangbori- Suwon346)	(Mihonhadaka- Chikurn Irabaki1, Frederickson-Zheda)
Min. GS	0.278	0.317	0.338	0.698	0.300
	(Cheongmaek- Suwon300)	(Suwon18- Suwon300)	(Baekdong- Suwon271)	(Suwon346- Doosan8)	(Kikahadaka- Diana)

[†]No. of banding patterns per primer = Avg. no. of banding patterns (marker genotypes) per primer

[‡]Avg. PIC = $\sum PIC_i / \text{Total no. of primers}$

[§]GS = Genetic similarity values calculated by simple matching coefficient method

Simple matching coefficient = (Number of matches) / (Number of matches + Number of unmatches)

Table 3. Polymorphic information content (PIC) values of 30 primers for six different barley germplasm pools.

Primer	Hulled barley (Korea)	Hulless barley (Korea)	Malting barley (Korea)	Hulled Variety (Korea)	Hulless Variety (Korea)	Foreign germplasm
opA19	0.20	0.15	0.20	0.08	0.00	0.65
opAA11	0.35	0.39	0.42	0.59	0.48	0.43
opB05	0.83	0.78	0.60	0.78	0.72	0.78
opB20	0.49	0.49	0.18	0.44	0.49	0.28
opC06	0.66	0.39	0.48	0.69	0.39	0.55
opC13	0.81	0.87	0.79	0.76	0.85	0.88
opC14	0.72	0.64	0.59	0.67	0.44	0.68
opD08	0.66	0.30	0.67	0.76	0.22	0.68
opD12	0.50	0.45	0.20	0.36	0.28	0.66
opE07	0.26	0.43	0.18	0.20	0.49	0.23
opE17	0.74	0.72	0.60	0.74	0.69	0.68
opF01	0.92	0.89	0.54	0.89	0.77	0.86
opF16	0.48	0.47	0.32	0.48	0.41	0.50
opG04	0.57	0.59	0.34	0.60	0.54	0.70
opH05	0.60	0.61	0.18	0.63	0.65	0.49
opN11	0.35	0.04	0.50	0.35	0.00	0.35
opP08	0.73	0.82	0.00	0.70	0.76	0.79
opR04	0.93	0.91	0.82	0.90	0.85	0.92
opS09	0.62	0.53	0.56	0.55	0.52	0.75
opT20	0.68	0.47	0.60	0.73	0.46	0.61
opU01	0.48	0.49	0.00	0.50	0.44	0.44
opU07	0.73	0.75	0.58	0.65	0.47	0.77
opV14	0.79	0.74	0.58	0.75	0.50	0.83
opW16	0.39	0.48	0.32	0.34	0.27	0.50
opX03	0.81	0.80	0.56	0.78	0.76	0.74
opX20	0.64	0.73	0.20	0.66	0.69	0.64
opY10	0.86	0.57	0.65	0.88	0.45	0.78

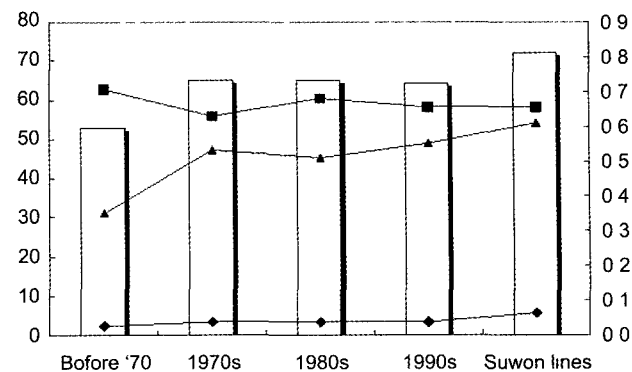
genomic loci (Table 3). The PIC scores ranged from 0.2 to 0.93 across the RAPD loci with an average of 0.62 for the hulled barley pool. However, the hulless barley pool had relatively low mean PIC (0.57) with a range of 0.04 - 0.91. As shown in Table 3, such primers as opR04, opF01, opB05, and opC13 had PIC scores of more than 0.8. Two randomly selected genotypes might be polymorphic with a probability of > 0.8 at these RAPD loci. However, relatively low polymorphism was detected at the genomic loci represented by such primers as opA19, opE07, opN11, and opAA11. Interestingly, almost no polymorphism was observed at opN11 in the hulless barley pool as compared to the hulled barley pool. Considering that PIC indicates the degree of genotypic or allelic variation at a random locus, the calculated scores in this study suggested that the hulled barley pool was more diverse than the hulless barley pool.

As for the malting barley pool, very low polymorphism was observed, and might be affected by the small number of accessions analyzed (Table 2). The level of genetic variation of two-rowed malting barley pool could not be compared with those of other pools due to the bias resulting from disparities of sample size. As expected on the basis of geographical origins and breeding background, the local malting barley varieties had significantly lower genetic variation than the other two-rowed barley in the exotic germplasm pool. More number of alleles were found in the foreign cultivars when the 2-rowed barley pool was examined. For example, 11 bands present in the 2-rowed exotic barley pool were not observed in the domestic malting barley varieties. Only five bands were specific to the domestic malting barley pool when compared to the 2-rowed exotic barley pool.

Patterns and temporal trends in the genetic diversity of the Korean barley cultivars and breeding lines were assessed over time. As shown in Fig. 2, according as time passes from 1970s to 1990s, the parameters of genetic diversity had a tendency to increase such as numbers of polymorphic bands or banding patterns, PIC, and genetic distance coefficient (1-GS). The increasing diversity has been apparent in both pools since 1980s. In particular, this upward slant was quite clear and significant for the hulless barley pool.

The average similarity coefficient among accessions over all germplasm pools was 0.628 with a range from 0.278 to 0.986. The range of similarity coefficient was larger in the hulled barley pool (0.317-0.986) than in the hulless barley pool (0.338-0.972). Based on the genetic similarity matrix of 13,530 genotype pairs calculated from RAPD analysis, all individuals of 165 accessions were clustered into two major groups I and II and several small subgroups within each group (Table 1). Fig. 3 illustrates the summary of dendrogram of 165 accessions trimmed at the genetic similarity

(a) Hulled barley pool



(b) Hulless barley pool

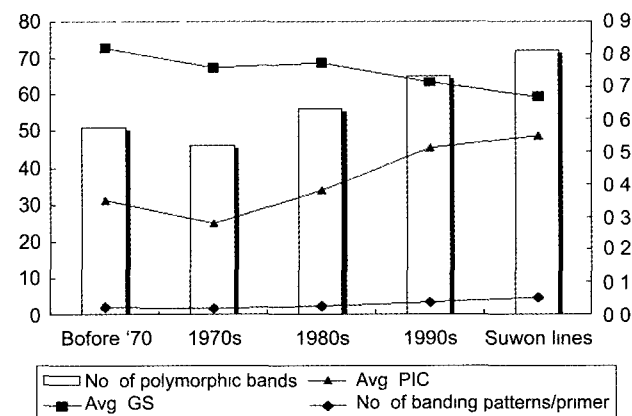


Fig. 2. Changes of genetic diversity over the periods in hulled (above) and hulless (below) barley varieties released until 1990s and Suwon-breeding lines

coefficient of 0.73 (please contact the corresponding author if the full description of dendrogram is required). Most of the 6-rowed hulled and hulless barley cultivars and lines belonged to the first major group I and were scattered within the group without regard to the presence of husk. This pattern of clustering became quite apparent when Suwon-breeding lines developed since 1990s were added in the analysis. On the other hand, as shown in Figs. 4 and 5(b), most of hulless barley varieties gathered in a group of 15 accessions when the cluster analysis was conducted with the released varieties only. The average value of genetic similarity estimates among 15 hulless barley varieties in this group was 0.807 which was greater than the average (0.676) of over all hulless barley pool. The trend of hulless barley varieties united into a group indicated that they were genetically very closely related since most varieties in this group were developed in the Honam Agricultural Research Institute in Iksan, Korea. However, recently developed hulless barley cultivars and breeding lines had more broad genetic base by

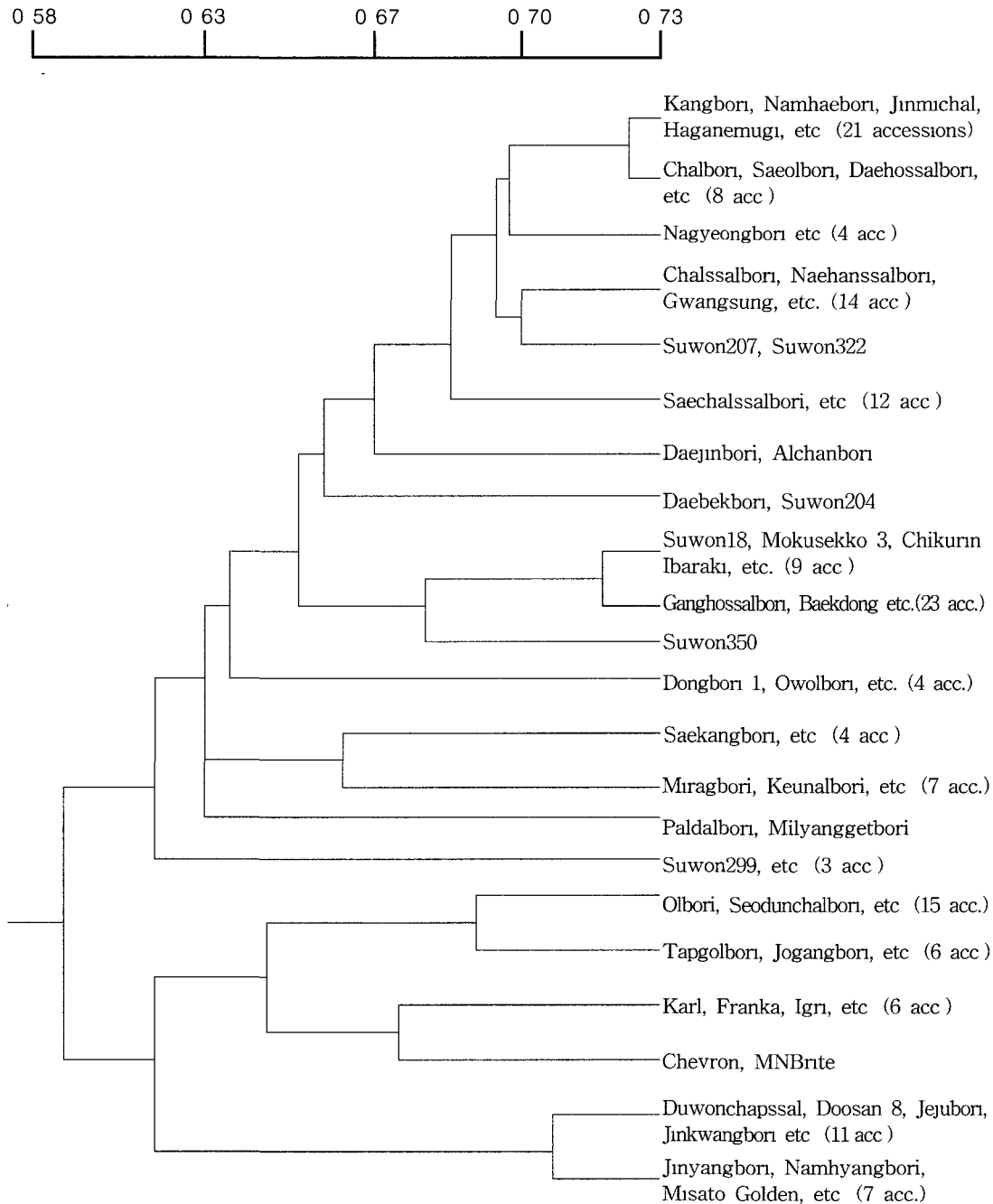


Fig. 3. Dendrogram trimmed at the genetic similarity (GS) coefficient of 0.73 resulting from cluster analysis of the RAPD-based GS matrix among 165 barley accessions.

selection followed by hybridization with hulled barley and other exotic germplasm for the improvement of the quality and biotic/abiotic resistance. Accordingly, no distinct grouping of accessions with specific phenotypes is observed when newly developed breeding lines are analyzed as shown in Fig. 3. Clustering results of the individual pools of released barley varieties are shown in Fig. 5.

According to the result of cluster analysis, it was typical

that a few accessions belonged to specific subgroups were shared by same or similar breeding background. Some of these subgroupings were resulted from the contribution of core accessions as major parents to the development of various descendent cultivars. Some of these core accessions are shown in the trimmed dendrogram (Fig. 3). For example, 'Haganemugi', a six-rowed hulled barley cultivar from Japan, was an important breeding parent extensively used

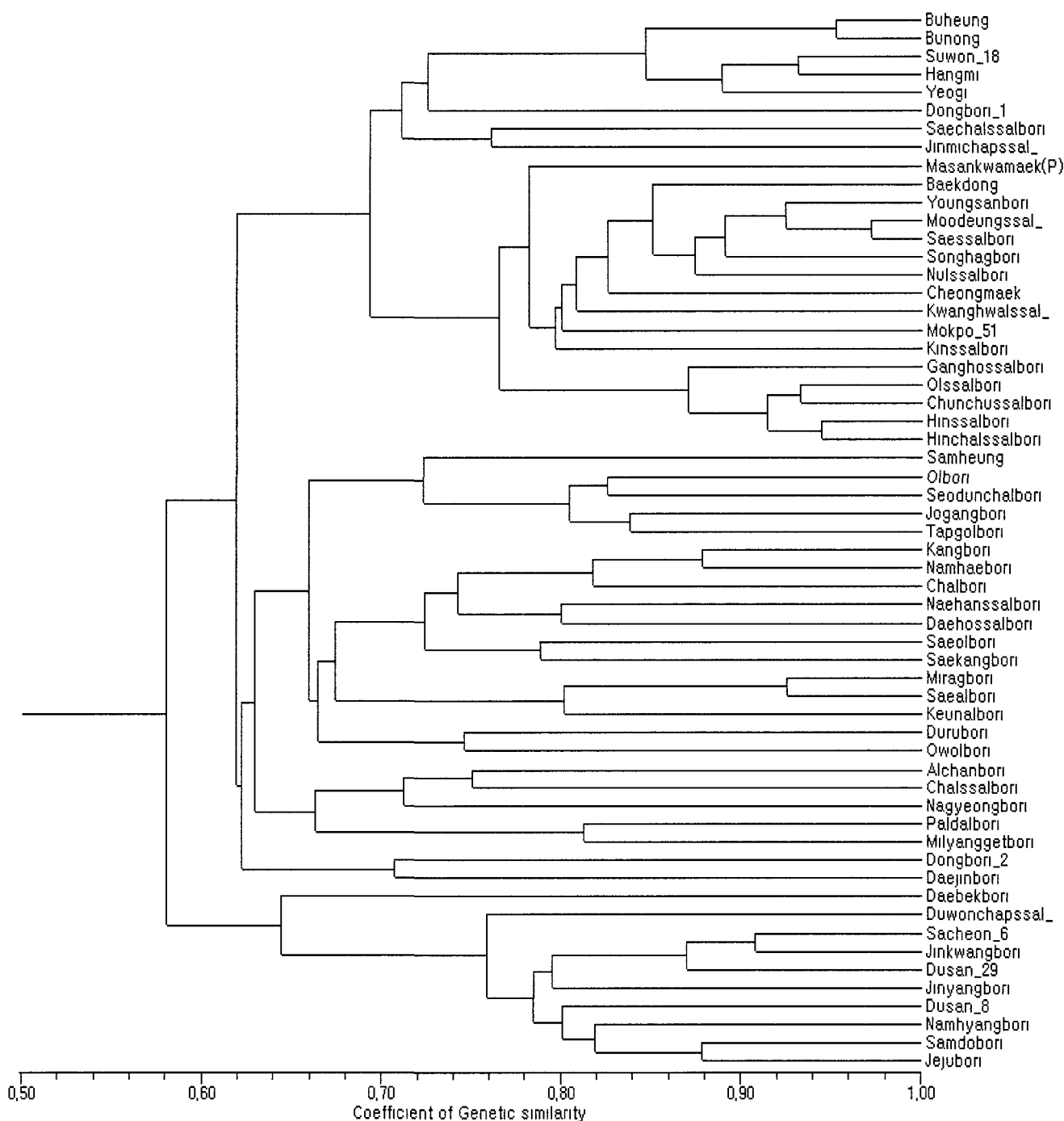


Fig. 4. Dendrogram resulting from cluster analysis of the RAPD-based GS matrix among 58 varieties of hulled, hulless and malting barleys released in Korea.

for the improvement of lodging resistance for hulled and hulless barley varieties in Korea in 1970~80s (Jong & Park, 2000a, b). As expected, it is the core germplasm in the tree of the first major group (I) in the dendrogram (Fig. 3). The average genetic similarity of 'Haganemugi' was 0.706 with the pool of 28 hulled varieties and 0.671 with the pool of 22 hulless varieties. Since 'Haganemugi' is known to have a

resistance gene (*rym3*) to barley yellow mosaic virus disease, the value of this cultivar has been reviewed including its progeny varieties such as 'Kangbori', 'Chalbori' and 'Namhaebori'.

The old varieties of covered barley developed before 1970s such as 'Bunong', 'Buheung', 'Yeogi', 'Hangmi' and 'Suwon 18' were closely related each other in the dendro-

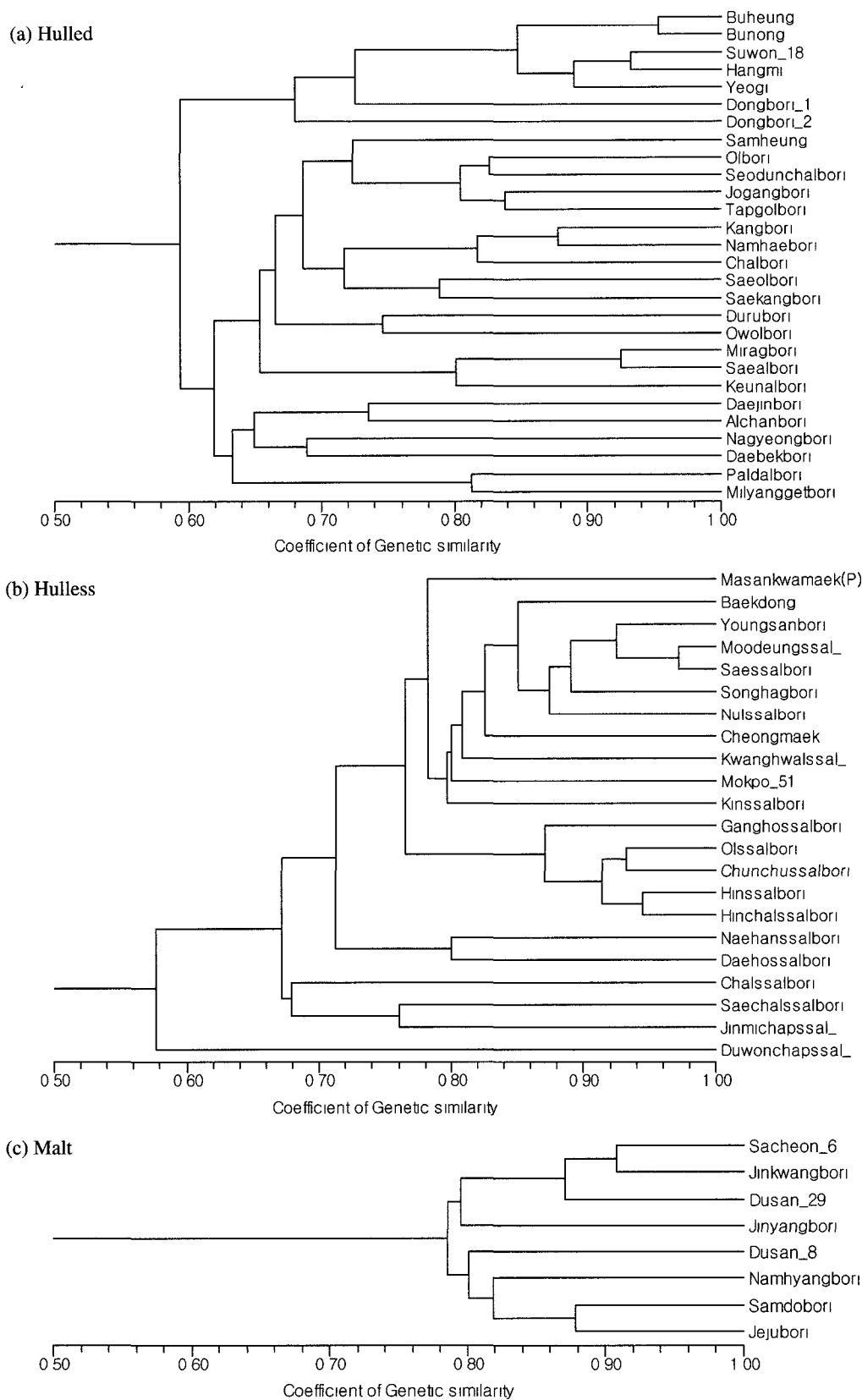


Fig. 5. Dendrogram resulting from cluster analysis of the RAPD-based GS matrix of (a) hulled, (b) hulless and (c) malting barley varieties released in Korea

gram (Figs. 4, 5a). This phase of grouping was not changed even if more accessions of recently developed lines were added in the analysis. This was because a parent for the first four old varieties was 'Suwon 18' that was developed in the cross between a Japanese introduction cultivar, 'Kinai Sekitori 3' and a Korean landrace, 'Yeongwolyukgak'.

Most of the two-rowed barley varieties and lines mainly used for malts belonged to a subgroup in the second main group. This clustering suggests that two-rowed barley is genetically distant from 6-rowed barley and is genetically closely related within a pool. This result of classification has already reported in other studies of barley germplasms using different DNA markers (Kim *et al* , 2002).

Exotic germplasms with geographical origins of Western world were included in this study to compare genetic relationships with Korean barley. As shown in the dendrogram (Fig. 3), they kept some distance with Korean barley accessions in the different subgroups. These foreign cultivars were important for improvement of Korean barley cultivars not only as the donor parents of useful characteristic natures such as resistance against barley yellow mosaic virus and scab (*Fusarium head blight*) but as the invaluable genetic resources to broaden genetic diversity.

Only limited number of intra- or inter-gene pool comparisons have been attempted in this study. The significant state of reduced genetic diversity due to the extensive hybridization among the elite breeding parents did not appear notably for Korean barley. In spite of presence of core parental genotypes in the clustered trees and close kinship among some specific materials in our studies, it is expected that genetic diversity of Korean barley pool may be maintained and enhanced via the qualitative shift by a steady build-up of valuable alleles for better phenotypes not by intentionally quantitative increase of genetic variation. It is still doubt whether DNA marker-based genetic diversity estimates help plant breeders to decide suitable genotypes to cross and to predict their heterosis regarding yield potential. We have tested how much molecular diversity estimates predict the yield potential for some crosses of barley genotypes, and the data will be analyzed. Though, the knowledge of genetic diversity and relationships between genotypes is important for the conservation of crop genetic resources and their exploitation for modern plant breeding and agricultural practices.

In addition, our RAPD data may be valid for identification of genotypes as DNA fingerprinting. DNA fingerprinting technique using reproducible polymorphic bands of RAPD access many anonymous chromosomal regions simultaneously and is quite efficient to access genetic diversity in many barley cultivars (Giese *et al* , 1994; Kochieva *et al* , 2001; Molnar *et al* , 2000). Several previous studies have

compared the utility of different DNA marker systems for the analysis of genetic diversity. Most of these showed that RAPD provided similar genetic relationships with other markers including RFLP, SSR, and AFLP even if different level of genetic heterozygosity was detected (Russell *et al* , 1997). From these results, RAPD is still one of the valid and popular DNA marker techniques when its easiness and simplicity for manipulation and low experimental cost are considered as compared to others. However, the overriding disadvantage of RAPD should be carefully manipulated when used as analytical marker tool because of its poor quality of genetic information and sensitiveness and variability to changes in reaction conditions.

REFERENCES

- Anderson, J. A., Y. Ogiwara, M. E. Sorrells, and S. D. Tanksley. 1992. Development of a chromosomal arm map for wheat based on RFLP markers. *Theor. Appl. Genet.* 83(8) : 1035-1043.
- Baek, H. J., S. J. Park, H. H. Kim, M. S. Yoon, S. Y. Lee, and W. Y. Choi. 2000. Changes of morphological traits according to period in Korean barley cultivars. *Korean J. Breed.* 32(4) : 356-362.
- Dahleen, L. S. 1997. Mapped clone sequences detecting differences among 28 North American barley cultivars. *Crop Sci.* 37(3) : 952-957.
- Giese H, A. G. Holm-Jensen, H. Mathiassen, B. Kjaer, S. K. Rasmussen, H. Bay, and J. Jensen. 1994. Distribution of RAPD markers on a linkage map of barley. *Hereditas* 3 : 267-273.
- Graner, A., W. F. Ludwig, and A. E. Melchinger. 1994. Relationships among European barley germplasm. II. Comparison of RFLP and pedigree data. *Crop Sci.* 34(5) : 1199-1205.
- Jang, D. H., S. J. Suh, S. B. Baek, J. G. Kim, and J. H. Nam. 2001. Efficiency of morphological traits and DNA markers in classification of Korean barley cultivars. *Korean J. Breed.* 33(4) : 300-305.
- Jong, S. K. and M. W. Park. 2000a. Cluster analysis of Korean six-row covered barley varieties based on coefficient of parentage. *Korean J. Breed.* 32(3) : 205-210.
- Jong, S. K. and M. W. Park. 2000b. Genetic diversity in naked barley varieties based on coefficient of parentage. *Korean J. Breed.* 32(4) : 303-308.
- Kim, H. S., K. G. Park, S. B. Baek, S. J. Suh, and J. H. Nam. 2002. Genetic diversity of barley cultivars as revealed by SSR marker. *Korean J. Crop Sci.* 47(5) : 379-383.
- Kochieva E. Z., S. V. Gorunova, and A. Pomortsev. 2001. An RAPD-marking of genomes of representative *Hordeum* species. *Genetika* 8 : 1088-1094.
- Molnar S. J., L. E. James, and K. J. Kasha. 2000. Inheritance and RAPD tagging of multiple genes for resistance to net blotch in barley. *Genome* 2 : 224-231.
- NCES (National Crop Experiment Station). 1998. Research achievement in winter cereal crops (1987-1991). R.D.A.
- Petersen, L., H. Ostergard, and H. Giese. 1994. Genetic diversity

- among wild and cultivated barley as revealed by RFLP. *Theor Appl Genet.* 89(6) : 676-681.
- Rohlf, F. J. 1992. NTSYS-pc, Numerical taxonomy and multivariate analysis system. ver. 1.7. Exter Publ. Setauket. New York. USA.
- Russell, J. R., J. D Fuller, M. Macaulay, B. G Hatz, A. Jahoor, W. Powell, and R. Waugh. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. *Theor. Appl Genet.* 95 : 714-722.
- Saghai-Marouf, M. A., K. M. Soliman, R. A. Jorgensen, and R. W. Allard. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci USA.* 81 : 8014-8018.
- WBRI (Wheat and Barley Research Institute). 1987. Research achievement and new direction in winter cereal crops. R.D.A.