Plant Res. 4(3) 121~129(2001)

Genetic Diversity among the Genera Allium in Mongolia Based on Random Amplified Polymorphic DNA (RAPD) Analysis

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ABSTRACT

Intraspecific genetic diversity of sixteen accessions of Mogolian *Alliums* including fifteen species was investigated using randomly amplified polymorphic DNA (RAPD) analysis. Twenty three out of forty primers revealed scorable polymorphism. A total of 440 RAPD markers were generated on the 16 accessions of Mongolian *Alliums*. Among 440 RAPDs assayed, 439 were polymorphic with a mean polymorphic rate of 99.7%. Unweighted pair-group method using an arithmetic average (UPGMA) cluster analysis using RAPD data separated the 16 *Allium* accessions into two broad groups at similarity index 0.70. The clustering of the species was closely related with previous classification between *A. altaicum* and *A. fistulosum*. In addition, a high genetic similarity was showed between *A. cepa* and *A. tagar*.

Key Words : *Allium,* Cluster analysis, Genetic diversity, Onion, Polymorphism, RAPD

INTRODUCTION

Allium has been grown for many centuries for their characteristic, pungent flavor and medicinal properties. However, only a few of these are important as food plants, notabaly onion, garlic, chives, leek and rakkyo (Fenwick and Hanley, 1985). *Allium* crops are an important crop worldwide, and generally used food and fodder in Mongolia (Table 1). Despite the major position of *Alliums* as a vegetable crop, a little genetic information is avaliable for them (Havey, 1991; Wilkie *et al.*, 1993).

Allium is a large genus with approximated 600 species and contains several major agricultural crops including the bulb onion (*Allium cepa*), shallot (*A. cepa syn. A. ascalonicum*), Japanese bunching or Welsh onion (*A. fistulosum*), chives (*A. schoenoprasum*), garlic (*A. sativum*) and leek (*A. ampeloprasum syn. A. porrum*). *Alliums* based on morphological criteria, crossability, and karyotype, were similarly divided as four sections (Traub, 1968). However, difficulties arise because there are relatively few morphological characters to base on a classification system, and because strong barriers to crossing separately, even if morphologically similar species are. Recently, it has been suggested that there could be a role for genetic markers in systematic studies (Havey, 1991),

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Table 1. List *of Allium* accessions, sampled sites and general uses in Mongolia.

Accession no.	*Allium* species	Source[a]	Remarks
1	*A. chinensis*	Darkhan-Uul	C[b], Food
2	*A. fistulosum*	Darkhan-Uul	C, Food
3	*A. cepa*	Selenge	C, Food
4	*A. tagar*	Middle Gobi	C, Food
5	*A. altaicum*	Darkhan-Uul	W[c], Food and fodder
6	*A. altaicum*	Bayank-hongor	W, Food and fodder
7	*A. polyrrhizum*	Khovd	W, Fodder
8	*A. mogolicum*	Gobi-Altai	W, Food and fodder
9	*A. odorum*	Baruun Kharra	W, Food and fodder
10	*A. senescens*	Bornuur Tov	W, Food and fodder
11	*A. anisopodium*	Bornuur Tov	W, Food and fodder
12	*A. maximovich*	Khentii	W, -
13	*A. prostratum*	Khubsugul	W, Food and fodder
14	*A. obliquum*	Darkhan-Uul	W, Food and fodder
15	*A. bidentatum*	Baruun Kharaa	W, Fodder
16	*A. leucocephalum*	Khubsugul	W, Food and fodder

[a]Regions from where the genotypes were collected.

[b]Cultivar.

[c]Wild species.

cytogenetic studies (Peffley and Currah, 1988; Peffley and Mangum, 1990; Ricroch *et al.*, 1992), protein (Peffley *et al.*, 1985) and RAPD studies (Heo *et al.*, 1998; Kim *et al.*, 1997; Wilkie *et al.*, 1993) of the species of Alliums. However, RAPD analysis using Mongolian *Alliums* has not been reported so far.

The aim of our research is to investigate genetic relationship and to identify molecular markers among Mongolian *Alliums* as revealed by RAPD analysis.

MATERIALS AND METHODS

Plant materials

Sixteen *Allium* accessions selected from Mongolia were used (Table 1, Fig. 1). Seeds of the *Alliums* were sowed in pot (15 ㎝ in diameter) and germinated at 25 ±2℃ with a 16-h daylength. The leaves of 16 accessions were sampled from plants grown in the pot and cut just before DNA isolation.

DNA isolation

Total genomic DNA was extracted from freshly harvested young leaves using the protocol of Dellaporta *et al.* (1983) with minor modifications. Leaves per each species (0.1 g) were ground in liquid nitrogen and then extraction buffer (0.2 M Tris-HCl, pH 8.0, 0.05 M EDTA, pH 8.0, 0.5M NaCl, 0.5% SDS) added. An equal volume of chloroform : isoamyl alcohol (24:1) was immediately added, and the solution was mixed and incubated on ice for 5 min. After centrifugation at 13,000 g for 5 min, the supernatant was collected, into

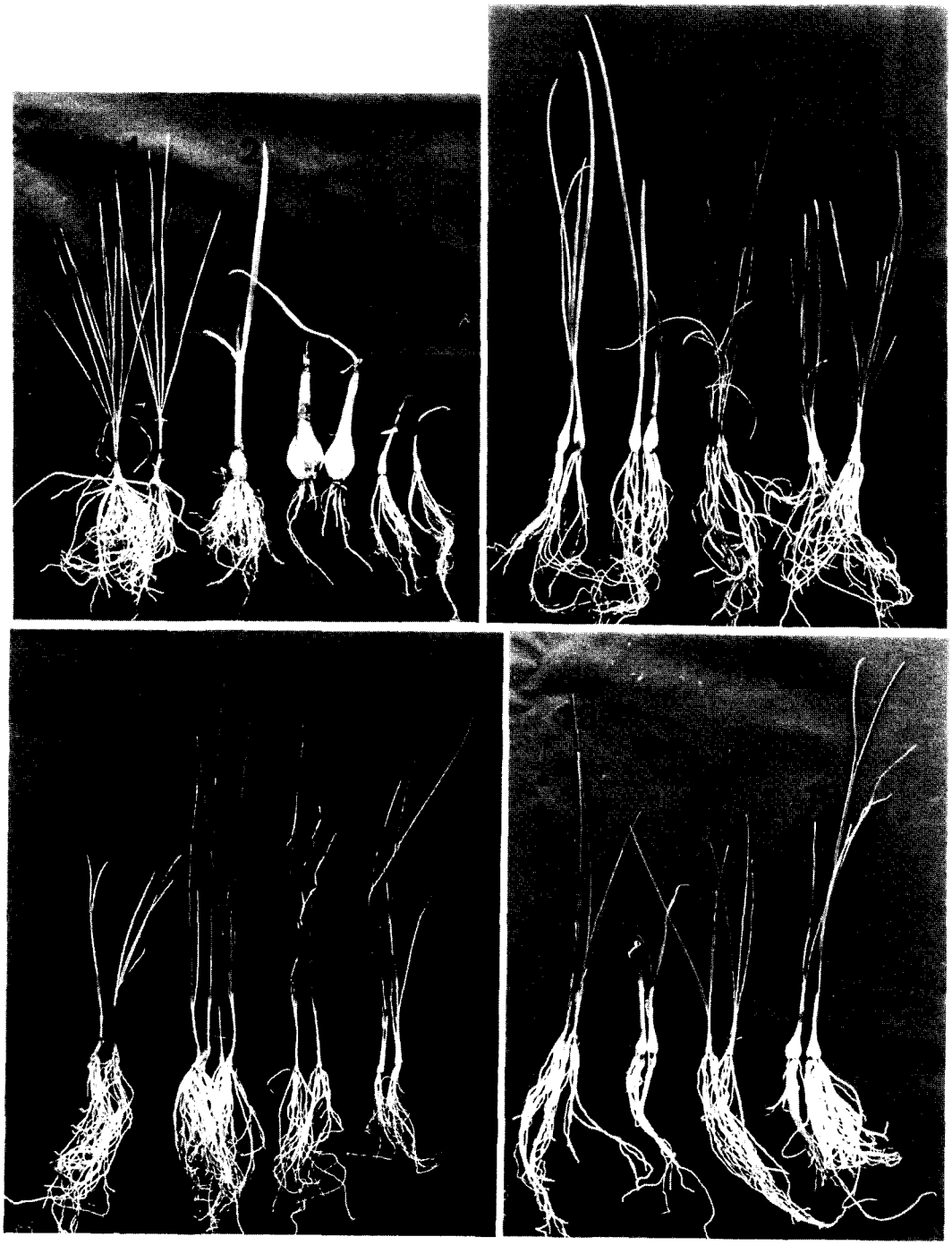

Fig. 1. Morphological features of *Alliums* used in this experiment. 1 : *A. chinensis*, 2 : *A. fistulosum*, 3 : *A. cepa*, 4: *A tagar*, 5: *A. altaicum*, 6 : *A. altaicum*, 7 : *A. polyrrhizum*, 8 : *A. mogolicum*, 9 : *A. odorum*, 10 : *A. senescens*, 11 : *A anisopodium*, 12 : *A. maximovich*, 13 : *A. prostratum*, 14 : *A. obliquum*, 15 : *A. bidentatum*, 16 : *A. leucocephalum*

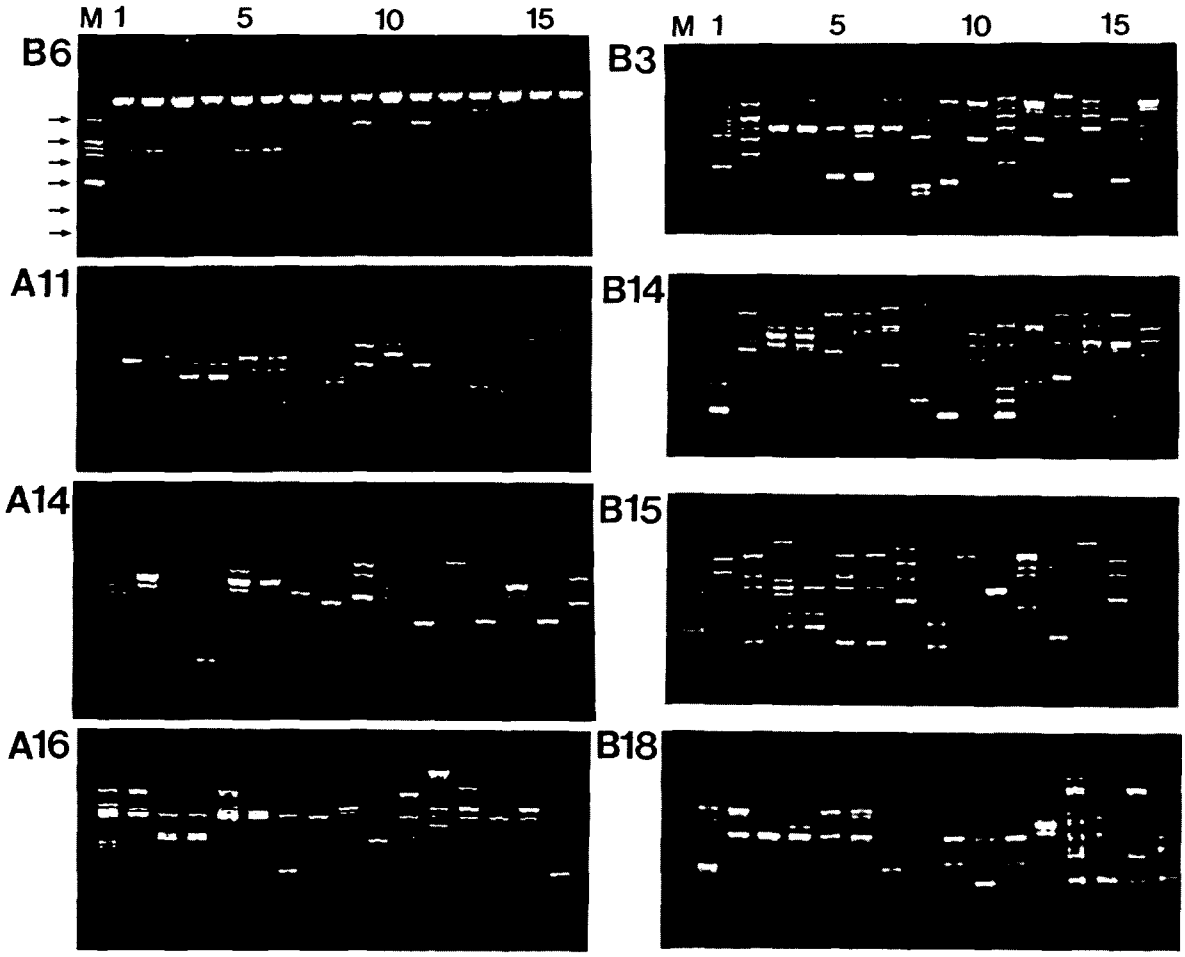

Fig. 2. RAPD profiles of 16 Mongolian Allium collections generated by 10-mer primer. Numbers are accession number of 16 collections shown in Table 1. M: Ladder DNA marker. Arrows indicate molecular weight 1,500, 1,000, 700, 500, 300 and 200 bp, respectively from upper to bottom.

which 2 volumes of ethanol were added, mixed and kept in a deep freezer (-80℃) for one hour. After centrifugation at 13,000 g for 5 min, the supernatant was removed. The DNA pellet was washed twice in 70% (v/v) ethanol, dried in a vacuum dryer, and resuspended in 1×TE buffer containing 10 $\mu g/ml$ RNase. The total genomic DNA was observed by electrophoresis on a 0.8% (w/v) gel and quantified by measuring absorbance at 260 nm by a spectrophotometer (Ultraspec^R 2000, Pharamacia Biotech).

DNA amplification

Two set of 20 random 10-mer primers purchased from Operon Technologies Inc. (Alameda Calif., USA) were used as single primer for the amplification of RAPD sequences (Table 2). DNA from 16 plant of *Alliums* was subjected to RAPD analysis following the conditions of Wilkie *et al.* (1993) with modifications. Amplification reactions were performed in 12 μl volumes containing 200 μM of each dNTP, 50 pmoles of primer, 500 ng genomic DNA, 1.5 mM $MgCl_2$, 1×

Table 2. Nucleotide sequences, G+C contents and number of detectable polymorphic bands of primers used.

Primers	Sequences (5' -3')	G+C contents (%)	Total bands	Polymorphic bands	Polymorphism rate (%)
A-01	GAGGCCCTTC	70	25	25	100
A-02	TGCCGAGCTG	70	17	17	100
A-05	AGGGGTCTTG	60	22	22	100
A-06	GGTCCCTGAC	70	22	22	100
A-07	GAAACGGGTG	60	13	13	100
A-11	CAATCGCCGT	60	24	24	100
A-12	TCGGCGATAG	60	17	17	100
A-14	TCTGTGCTGG	60	16	16	100
A-16	AGCCAGCGAA	60	17	17	100
A-17	GACCGCTTGT	60	18	18	100
A-19	CAAACGTCGG	60	20	20	100
B-02	TGATCCCTGG	70	17	17	100
B-03	CATCCCCCTG	70	21	21	100
B-04	GGACTGGAGT	60	17	17	100
B-05	TGCGCCCTTC	70	19	19	100
B-06	TGCTCTGCCC	70	6	5	83
B-07	GGTGACGCAG	70	19	19	100
B-10	CTGCTGGGAC	70	21	21	100
B-14	TCCGCTCTGG	70	20	20	100
B-15	GGAGGGTGTT	60	25	25	100
B-18	CCACAGCAGT	60	22	22	100
B-19	ACCCCCGAAG	70	25	25	100
B-20	GGACCCTTAC	60	16	16	100
	Total		440	439	99.7%

PCR buffer and 0.5 unit of *Taq* DNA polymerase (Takara, Japan). The amplifications were carried out in a Perkin Elmer Cetus 9700 thermocycler using the following amplification profile: 1 cycle at 94℃ for 2 min and 30s, followed by 40 cycles of 94℃ for 30s, 40 ℃ for 30s and 72℃ for 1 min with a final extension at 72℃ for 5 min. PCR products were denatured and resolved by electrophoresis on 1.5% agarose gels and visualized by ethidium bromide staining (Sambrook *et al.*, 1989).

Data analysis

Only clear and unambiguous DNA bands were included in the analysis. Markers were scored for presence (1) and absence (0) of the band in RAPD analysis. Bands of different electrophoretic mobilities were assumed to be non-allelic, while bands of the same mobility were assumed to be allelic. A pairwise similarity matrix was calculated using the Jaccard Coefficient. UPGMA cluster analysis was used to

Table 3. Similarity matrix of genetic distances among 16 Mongolian Allium. Numbers are accession number of 16 collections shown in Table 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.000															
2	0.684	1.000														
3	0.690	0.675	1.000													
4	0.686	0.675	0.945	1.000												
5	0.684	0.843	0.692	0.679	1.000											
6	0.688	0.809	0.722	0.713	0.885	1.000										
7	0.701	0.673	0.713	0.692	0.699	0.711	1.000									
8	0.677	0.675	0.703	0.682	0.688	0.705	0.730	1.000								
9	0.726	0.673	0.705	0.701	0.699	0.720	0.694	0.688	1.000							
10	0.699	0.679	0.699	0.711	0.692	0.709	0.722	0.715	0.718	1.000						
11	0.737	0.650	0.715	0.699	0.667	0.667	0.675	0.665	0.803	0.673	1.000					
12	0.692	0.669	0.688	0.684	0.635	0.677	0.690	0.696	0.699	0.692	0.696	1.000				
13	0.686	0.667	0.703	0.690	0.667	0.692	0.696	0.703	0.701	0.715	0.686	0.696	1.000			
14	0.726	0.703	0.709	0.701	0.699	0.715	0.728	0.696	0.690	0.701	0.692	0.728	0.718	1.000		
15	0.711	0.658	0.711	0.703	0.667	0.679	0.718	0.711	0.726	0.720	0.720	0.709	0.873	0.730	1.000	
16	0.745	0.692	0.724	0.711	0.701	0.722	0.747	0.749	0.739	0.741	0.728	0.735	0.754	0.760	0.771	1.000

identify genetic variation patterns among the Allium genotypes. Cluster analysis was performed using NTSYS-PC (NTSYS for Numerical Taxonomy Systems) software version 2.0.

RESULTS AND DISCUSSION

Extent of polymorphism revealed in the *Allium*

Among the 40 RAPD primers tested on the 16 *Allium* samples, clear and scorable products were obtained from 23 primers that produced 440 DNA fragments, of which 439 were polymorphic (Table 2). Only one monomorphic band was obtained from primer B-06 (Fig. 2). The mean polymorphism rate was 99.7% and the mean number of bands per primer was 19.1. The total number of bands per primer ranged from 6 to 25, and the number of polymorphic bands per primer ranged between 5 and 25 (Table 2) with a size approximately ranging between 200 and 2,300 bp (Fig. 2). The total number of DNA bands generated in this study was greater than that observed in 41 accessions which revealed 7.5 bands per primer in intraspecific *Allium* plants (Kim *et al.*, 1997). This result may be due to the fact that polymorphism is higher in the extraspecific than in the intraspecific.

The level of polymorphism observed using RAPD primers indicates a remarkable amount of extraspecific genetic variation among the Mongolian *Allium* samples. The result with large amounts of variation at RAPD level was consistent with that tremendous genetic diversity exists in the genera *Alliums* (McCollum, 1976). This high levels of polymorphism allow for the selection of parents and the identification of true hybrids in breeding programs (Garcia *et al.*, 1998). The marker data may be a valuable tool for germplasm evaluation and for exploitation of heterosis in breeding

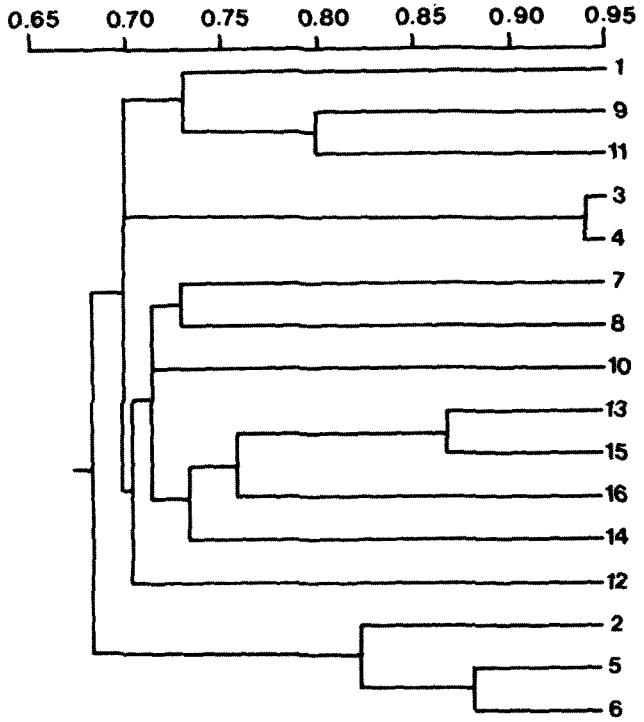

Fig. 3. Dendrogram generated by cluster analysis of genetic distance values given in Table 3 showing relationships between different *Allium* species. Relative branch lengths indicate relative genetic distances between species. Numbers are accession number of 16 collections shown in Table 1.

programs. Moreover, the polymorphism information obtained through RAPD analysis may be also useful to identify polymorphic primers for further studies in *Allium* genera.

Genetic similarity

Genetic similarities among the 16 *Alliums* ranged between 0.684 and 0.945 (Table 3), a distance that was lower than that reported by Kim *et al.* (1997) in intraspecific variations of *A. victorialis* species with a genetic distance ranging from 0.77 to 0.99. The RAPD dendrogram seperated the 16 entries into two broad groups ; Group Ⅰ, including 13 accessions, with 0.701 ~0.945 similarity indices (Fig. 3). And three sub-clusters at similarity index 0.701. Group Ⅱ, 3 accessions, with 0.826~0.885 similarity indices. Its high similarity indices arise because *A. fistulosum* (accession no. 2) is originated from *A. altaicum* (Wilkie *et al.*, 1993), and because accessions of no. 5 and 6 are the same species that grows naturally only in different localities (Table 1). Cytogenetic evidence of introgression of *A. fistulosum* (accession no. 2) into *A. cepa* (accession no. 3) has been reported (Peffley and Mangum, 1990), but their genetic similarity showed great distance each other. Strong interspecific crossing barriers existing between *A. cepa* and *A. fistulosum* reflected in the genetic distance between them. This is consistent with results of Wilkie *et al.* (1993). *A. tagar* (accession no. 4) is more closely related to *A. cepa* than *A. fistulosum* based on genetic similarity estimates. This

result showed compatibility between them according to their similar phenotype (Fig. 1). While *A. tagar* (accession no. 4) and *A. obliquum* (accession no. 14) are not under similar cluster, even though its phenotype is very similar. Furthermore, among three species having long-narrowish leaves, *A. prostratum* (accession no. 13) and A. bidentatum (accession no. 15) showed a high genetic similarity, but *A. polyrrhizum* (accession no. 7) is similar to *A. mogolicum* (accession no. 8) that has leek like long-flat leaf (Fig. 1, Fig. 3). Although some of exceptions exist, classification by the clustering corresponds with morphological phenotype of the *Alliums*. However, all of the *Allium* species that are similar to their phenotype did not group in the same cluster (Fig. 1, Fig. 3). Its reason might be due to fact that differences between morphological phenotypes and qualities, such as flavor and taste of the *Alliums* resulted in genetic diversity. This fact indicated that even the morphologically similar species might produce different flavor, or taste. For example, *A. obliquum* (accession no. 14) shows very similar phenotype, taste and smell to garlic, but just bulb is very similar to onion. This result indicates that taxonomic classsifications of the *Alliums* by its morphological characters are very difficult because of its tremendous genetic diversity (McCollum, 1976). It is suggested that not only morphological characters but also cytogenetical and molecular marker analysis such AFLP, RFLP were considered as classsification key. Nevertheless, one monomorphic band and some of species specific bands were obtained from this experiments (Fig. 2). These results will contribute to further studies in *Alliums*. Further study should be included an analysis of molecular markers such as species specific band or monomorphic band of Mongolian *Alliums*.

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(Recived 2001. 9.20)
(Accepted 2001.11.20)