Akd. Üniv. Zir. Fak. Derg., 10, 136-142, 1997

GENETIC ANALYSIS OF CERTAIN *ALLIUM* SPECIES WITH RAPD-PCR

Leyla AÇIK[1], Bülent SAMANCI[2], Mehmet YAPAR[3], Ayhan KUBAR[3]

Abstract: Random amplified polymorphic DNA (RAPD) markers were used to identify and distinguish between several Turkish *Allium* species: *A. neopolitanum, A. callidictyon, A. rupestre, A. scorodoprasum, A. affine* and *A. junceum*. The primers A10 were evaluated for their usefulness. Reactions with primer A 10 produced amplified band and the size of amplified DNA fragments ranged from 100 to 947 base pairs. Reactions with another group of 11 primers made possible the separation of some but not all species. Genetic distances between *A. neopolitanum, A. callidictyon, A. rupestre, A. scorodoprasum, A. affine* and *A. junceum* were calculated.

Key Words: RAPD-PCR, Genetic analysis, dendogram.

Bazı *Allium* Türlerinin RAPD-PCR ile Genetik Analizleri

Özet: RAPD belirleyicileri Türkiye'de bulunan *Allium* türlerini ayırmak ve belirlemek için kullanıldı: *A. neopolitanum, A. callidictyon, A. rupestre, A. scorodoprasum, A. affine* ve *A. junceum*. A10 primeri faydalılığı için değerlendirildi. A10 primeri ile reaksiyonlar amplifike olmuş band üretti ve amplifike olmuş DNA parçalarının büyüklüğü 100-947 baz çifti arasında değişti. Diğer 11 primer grubu ile reaksiyonlar bazı türlerin ayrışımını kolaylaştırdı. *A. neopolitanum, A. callidictyon, A. rupestre, A. scorodoprasum, A. affine* ve *A. junceum* arasındaki genetik uzaklıklar hesaplandı.

Anahtar Kelimeler: RAPD-PCR, Genetik analiz, Dendogram.

INTRODUCTION

The large genus *Allium* with perhaps as many as 600 species, is distributed throughout the Northern Hemisphere and is especially common in Europe and Western Asia. In Turkey, there are about 150 species of *Allium*. Some species are commonly cultivated as vegetables and some are of ornamental value (3).

The taxonomy of Allium undoubtedly is not an easy matter (2). Morphological differentiation is rather weak and it appears that at species level other disciplines such as palylonogy and cytology provide rather limited further information (8). However, other criteria have been developed to detect species identification such as isozymes (4) and RFLP (5, 9, 11,13). Isozymes and RFLP analyses are laborious and time consuming. Amplification conditions for RAPD analysis are similar to those used in a

1. Gazi Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Ankara
2. Akdeniz Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Antalya
3. Gülhane Askeri Tıp Akademisi, Viroloji ABD, Ankara

normal polymerase chain reaction except that one primer with an arbitrary sequence is used instead of two primers with sequences (10, 12).

Random amplified polymorphic DNA markers would be useful and have advantages for genetic and systematic studies in *Allium*. This study reports species identification of *A. callidictyon, A. rupestre, A. scorodoprasum, A. affine* and *A. junceum* using RAPD.

MATERIALS AND METHODS

Plant materials: *Allium* species selected from sections Molium (*A. neopolitanum*), *Allium* (*A. affine, A. scorodoprasum, A. junceum*), Cododprasum (*A. repestre*), Brevispatha (*A. callidiction*) were used. Plant species were kindly provided by Prof. Dr. Mehmet Koyuncu.

DNA extraction: *Allium* leaves were grounded in liquid nitrogen and DNA extracted according to the modified method of Açık & Samanci (1).

Primer synthesis: A set of 10mer Operon random oligonucleotide primers were provided by Fermentas company.

Amplification conditions: RAPD amplification were optimized. Reactions were performed in a volume of 100 μl containing 20 mM Tris-HCl (pH 8.8), 500 mM KCl, 0.8 % Nonidet P40; 25 mM MgCl2; 100 mM each of dATP, dCTP, dGTP, dTTP; 0.2 mM primer; 25 ng of Allium genomic DNA; and 1 U Taq polymerase (without BSA-MBI Fermentas) using TECHNE Progene Thermal cycler. Each cycle consisted of 30 sec at 94 0C, 30 sec at 72 0C for 45 cycles. Amplified DNA fragments were separated by electrophoresis through 1.0 % agores gel in 1XTAE buffer (7). Twenty microliters were loaded on the gel. Gels were stained with ethidium bromide and fragment patterns were photographed. Different fragments produced with each primer were numbered sequentially and presence or absence of fragments in each sample was scored and compared with each other (14).

Data analysis: Variability among species was expresses as similarity "S" calculated as $S= 2 X N_{AB} / N_A + N_B$ where N_{AB} is the total number of bands shared by individuals A and B, and N_A and N_B are the number of bands in individuals A and B, respectively. The genetic distance D is D=1-S. The genetic distances were calculated for each species pair. Cluster analysis and dendograms were constructed from the genetic distance data using SPSS computer program.

RESULTS AND DISCUSSION

RAPD-PCR techniques can be used in cultivar identification and phlogenetic studies which can help plant taxonomists in order to measure genetical distances among species. It would also allow a more quantitative assessment of genetic distances between species. Such an analysis, together with data from other methods, could thus be used to make a more accurate reconstruction of the genus *Allium* . These variations are, in most cases, polygenically inherited and reveal polymorphisms. The observation that complexity of RAPD profiles is independent

of the size of the genome is difficult to explain. In RAPD reactions, the composition of the amplification products is determined by a competition between potantial priming sites in the template rather than by the total number of priming sites available (6).

In this study, PCR techniques is used to identify *Allium* species. To find a suitable primer for species identifications, several primers were used to amplify genomic DNA from each *Allium* species. Some of the primers produced weak amplification with some species. Some others provided band patterns that allowed the separation of only one or two species. These primers were discarded. The primer A10 produced amplification patterns that distinguished between Allium species in this study. After repeated amplification with different individuals of each species gave the same amplification patterns, A10 primer could be considered species specific. Figure 1 and 3 shows the results of an experiment in which primer A1 used to amplify segments of genomic DNA from *A. neopolitanum, A. callidictyon, A. rupestre, A. scorodoprasum, A. affine* and *A. junceum.*

Several individuals representing each species from different origin were also assayed. Following amplification, DNA samples were analyzed by agorose gel electrophoresis.

The profile of the amplified products for each *Allium* species was compared in pair-wise fashion and allowed identification of species specific markers. In general, they shared the same profile with most of the primers tested. The numbers of bands in the profiles varied, depending on the species and individuals tested.

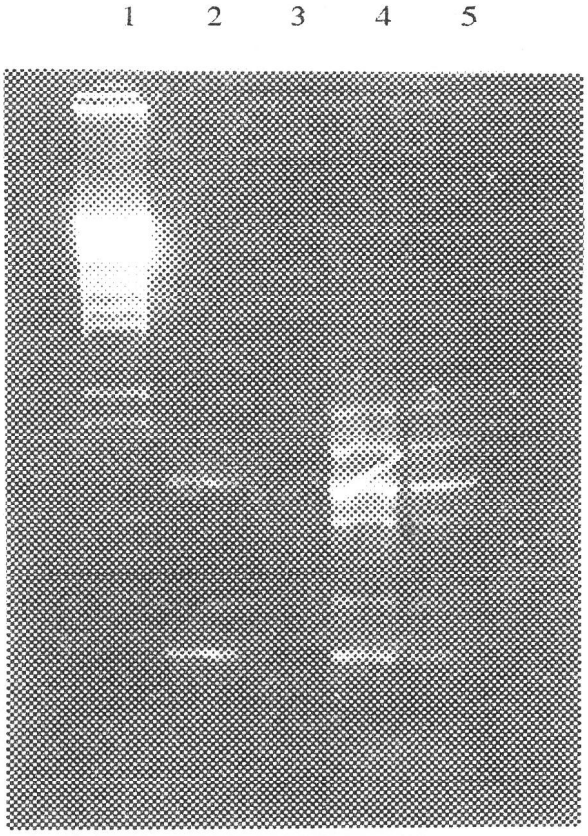

Figure 1. RAPD amplification products produced in *A. callidictyon* (line 2 and 3), *A. rupestre* (line 4 and 5 from left to right) with A1 primer. Line 1 is a DNA fragment cut with HindIII/EcoRI.

The band numbers ranged from 2 (*A. neopolitanum* and *A. callidictyon*) to 7 (*A. affine*). Most of the *Allium* species could be distinguished amplification profiles from primer 10. Within individuals of each species, there were identical amplification pattern except for *A. neopolitanum* but not

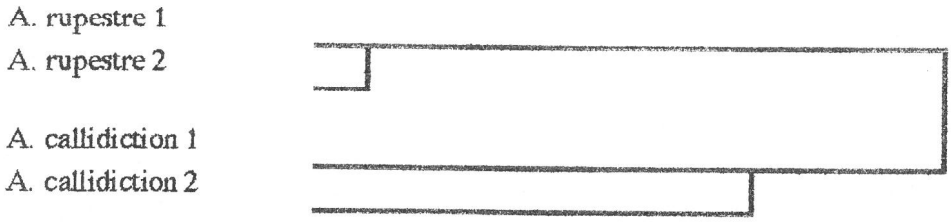

Figure 2. Dendogram generated by cluster analysis of genetic distance values given in Table 1 showing relationships among different *Allium* species. Relative branch lengths indicate genetic distance between species.

Table 1. Genetic distance values among species of *Allium* (*A. callidiction* and *A. rupestre*) calculated as described in Material and Methods.

Species	*A. callidiction-1*	*A. callidiction-2*	*A. rupestre-1*	*A. rupestre-2*
A. callidiction-1	0			
A. callidiction-2	0.50	0		
A. rupestre-1	0.56	0.78	0	
A. rupestre-2	0.56	0.78	0	0

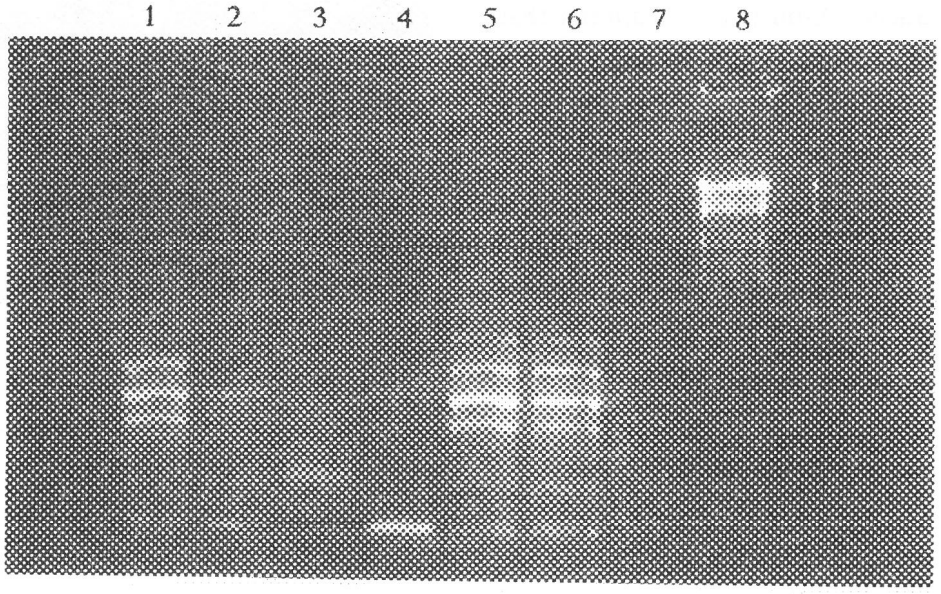

Figure 3. Polymorphic bands amplified DNA produced in *A. affine*(line 1), *A. neopolitanum* (line 2-4), *A. scorodoprasum*(line 5-6) and *A. junceum*(line 7 from left to right) with A1 primer. Line 8 is a DNA fragment cut with HindIII/EcoRI.

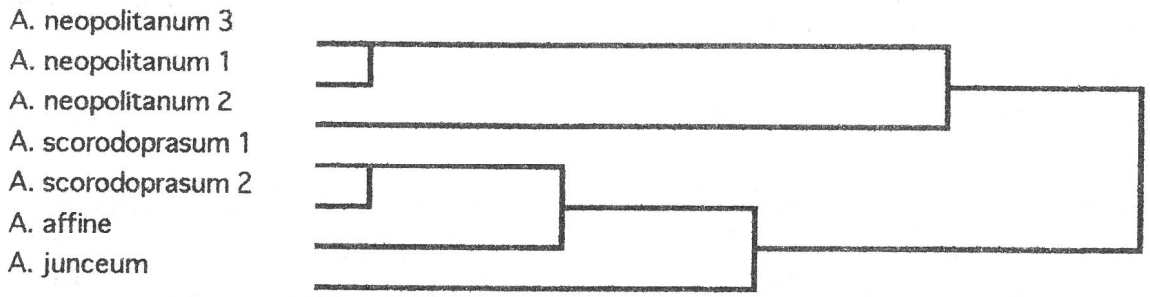

Figure 4. Dendogram generated by cluster analysis of genetic distance values given in Table 1 showing relationships among different *Allium* species. Relative branch lengths indicate genetic distance between species.

Table 2. Genetic distance values among species of *Allium* (*A. affine, A. neoplotanum, A. scrorodoprasum, A. junceum*) calculated as described in Material and Methods.

Species	*A. affine*	*A. neop-1*	*A. neop-2*	*A. neop-3*	*A. sco-1*	*A. sco-2*	*A. junceum*
A. affine	0						
A. neopolitanum-1	0.80	0					
A. neopolitanum-2	1	0.60	0				
A. neopolitanum-3	0.80	0.00	0.60	0			
A. scorodoprasum-1	0.23	0.56	0.75	0.56	0		
A. scorodoprasum-2	0.23	0.56	0.75	0.56	0	0	
A. junceum	0.60	0.67	1.00	0.67	0.56	0.56	0

identical banding patterns (Figure 3).

Polymorphic bands were scored as present and absent and the data were used to calculate genetic distance values among *Allium* species. Genetic distance values between *A. callidictyon* and *A. rupestre* are given in Table 1. The genetical distances within the individuals of *A. rupestre* was 0.0 based on banding patterns whereas, the distance between the individuals of *A. callidctyon* was 0.50 based on RAPD markers used in this study. The dendogram generated by cluster analysis of genetic distance values for the species shown in Figure 2. The distance between the individuals of *A. callidiction* was higher than the individuals of *A. rupestre.*

Table 2 shows the genetical distances among *A. nepolitanum, A. scorodoprasum, A. affine* and *A. junceum* using. There were no band differences between *A. scorodoprasum* individuals (distance =0.0). One of the individuals of *A. neoplotanum* differed from each other. (distance = 0.60). Genetic distances among species varied between 0.23 to 1.00, closest relative to further ones. The most closest relative of *A. affine* was *A. scorodoprasum* .

As a result, cluster analysis based on genetic distances generated dendograms indicating relationships between the *Allium* species used. Generated dendograms were in general agreement with the previously implied classification of genus *Allium*.. RADP-PCR can be used for species identification which was used to be done morphologically. Polymorphism revealed by this technique can also be used in evolutionary studies and be measured among species even the individuals within the species.

Cultivar identification and cultivar relatedness are important issues for breeders. The application of RAPD's seems very useful in this regard. If primers are chosen that are known to give highly polymorphic banding patterns in *Allium* only a few primers are needed to distinguish cultivars. Therefore, cultivars derived vegetatively from one cultivar can not be distinguished from each other using this technique. At interspecific level, a considerable degree of polymorphism was revealed in *Allium* by the RAPD technique and the polymorphism observed were successfully scored and used in common-band analyses similar to those applied in other crops using RFLP's and RAPD's. The values of genetic distance obtained and dendograms produced from them appear to be informative at indicating relationships between the species studied.

REFERENCES

1. AÇIK L., B. SAMANCI, F. DUMAN, F. UNAL. Polymorphism and Phylogenetic Relations among Turkish Species in The Genus *Allium* as Determined RAPD-PCR. Turkish Journal of Botany. In press. 1997.

2. BADR, A., T. ELKINGTON. Numeral Taxonomy of Species in *Allium*. New Phytol, 81, 401-364, 1993.

3. DAVIS, P. H. In Flora of Turkey and East Aegean Islands. Vol VIII. pp.360-364, 1984.

4. HADOCOVA,V.,J.SVACHULOVA E. KLOZOVA, HADOC, K. PITTEROVA. Use of Estrase Isoenzymes Revealed by Gel Isoelectric Focusing as and Aid in Chemataxonomical Study of the Genus Allium. Bio Plant, Praque. 259,36-42, 1983.

5. HANELT, P. In Onions and Allied Crops Vl. II. Botany, Physiology and Genetics. CRC Press Inc. Boca, Raton, Florida, pp.1-26, 1989.

6. RAFALSKI, I.A., S.V. TINGEY, J.G.K. WILLIAMS. RAPD Markers- a New Technology for Genetic Mapping and Plant Breeding. Ag. Biotech News Info.3, 645-648, 1991

7. SAMBROOK, J., F.F. FRITCH, T. MANIATIS. In Molecular Clonning. A Laboratory Manual. Second edition, Cold Spring Harbour, 1989.

8. STEARN, W. Notes on the Genus *Allium* in the Old World. Herbertia. 11, 11-30.1944.

9. TANKSLEY, S.D., N.D. YOUNG, A.H. PETERSON, M.W. NONIERBALE. RFLP Mapping in Plant Breeding New Tools for an Old Science. Biotechnolgy, 7:257-264, 1989.

10. WELSH, J., C. PETERSON, M. MCCLLELAND. Polymorphism Generated by Arbitrarily Primed PCR in the Mouse: Applications to Strain Identification and Genetic Mapping, Nucleic Acids Res. 19, 903-906, 1991.

11. WELSH, J., C. PRETZMEN, D. POSTIC, T. SAINT-GRIONS, G. BATONTON, M. MCCLELLAND. Genomic Fingerprinting br Arbitrarily Primed PCR Resolves *Borrelia burgdorferi* into Three Distinct Phyletic Groups. Int. Sys. Bacteriol. 42, 370-377, 1992.

12. WILKIE, S.E., P. G. ISAAC, J.R. SLATER. RAPD Markers for Genetic Analysis in *Allium*. Thor Appl. Genet. 86, 497-504, 1993.

13. WILLIAMS J.G., A.R. KUBELIK, K. LLIVAC, J.A. RAFALSKI, S. TINGEY. DNA polymporphism Amplified by Arbitrary Primers are Useful as Genetic Markers. Nucleic Acids Research. 18 (22), 6531-6535, 1990.

14. WOLF, K., J.P. RIJN. Rapid Detection of Genteic Variablity in *Chrisanthemun* Using Random Primers. Heridity. 71, 335-341, 1993.