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IDENTIFICATION OF FIVE *ALLIUM* SPECIES WITH RAPD MARKERS

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Abstract: Random amplified polymorphic DNA (RAPD) analysis was applied to *Allium* species in order to check the degree of polymorphism within the genus. Five species of *Allium, A. isauricum, A. myrianthum, A. curtum, A. ılgazasense* and *A. karamanoglu* were evaluated for variability using a set 10mer random primers. One of the primer revealed scorable polymorphism between *Allium* species. Variation in banding profiles between species were observed. These were applied to systematic studies within the genus. Ten band positions were scored. Genetic distances between species were calculated and cluster analysis were used for a dendogram showing phylogenetic relations among species studied.

Key Words: RAPD-PCR, *Allium*, *Allium* taxonomy, Phylogeny

RAPD İzleri ile 5 *Allium* Türünün Belirlenmesi

Özet: RAPD analizi cins içersinde polimorfizmim derecesini kontrol etmek için *Allium* türlerine uygulanmıştır. *Allium* 'un beş türü, *A. isauricum, A. myrianthum, A. curtum, A. ılgazasense* and *A. karamanoglu* şansa bağlı primerler kullanılarak varyasyonu belirlemek için değerlendirilmişlerdir. Primerlerden biri *Allium* türleri arasında sayılabilir polimorfizmi ortaya koymuştur. Band profilinde türler arasındaki varyasyonlar gözlenmiştir. Bunlar cins içersinde sistematik çalışmalara uygulanmıştır. 10 band pozisyonu sayılmıştır. Türler arasındaki genetik uzaklıklar hesaplanmış ve kluster analizi çalışılan türler arasındaki filogenetik ilişkileri gösteren dendogram için kullanılmıştır.

Anahtar Kelimeler: RAPD-PCR, *Allium*, Soğan taxonomisi, Filojeni

INTRODUCTION

Allium is a large and economically important genus representing as many as 600 species in the world and 150 species in Turkey. Some species are commonly

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cultivated as vegetables and some are of ornamental value. However, many are not well known at the present (2). Classification and identification of such a large genus has proved difficult and many ambiquities still remain (3). Despite the position of *Allium* is a vegetable crop, very little genetic information is available for it. Recently, polymorphic DNA markers have made a major contribution to plant genetic improvement (6, 7, 9). This technique is based on the amplification of random DNA sequences by polymerase chain reaction except that one primer with an arbitrary sequence is used for instead of two primers with sequences. The advantages of this technique are its ability to detect extensive polymorphisms, simplicity, rapidity and need for very small amounts of genomic DNA. In this study, we aim to identify genotypes of five *Allium* species and show the genetic distances by using RAPD markers.

MATERIALS AND METHODS

Plant materials: *Allium* species, *A. isauricum, A. myrianthum, A. curtum, A. ilgazasense* and *A. karamanoglu* which are kindly povided by Prof. Dr. Mehmet Koyuncu.

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

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

Amplification conditions: The DNA was amplified under similar condition to PCR with the exception that only a single primer was used that nucleotide order of the primer was random. In a previous experiment, the reaction conditions were optimized. Reactions were performed in a volume of 10 μl containing 20 mM Tris-HCl, 0.8 % Nonidet P40; 25 mM MgCl2; 100 μM each of dATP, dCTP, dGTP, dTTP; 0.2 μM primer; 25 ng of *Allium* genomic DNA and 1 U Taq polymerase (without BSA-MBI Fermentas) using TECHNE Progene Theremal Cycler. Each cycle consist of 30 sec at 94 °C, 30 sec and 72 °C. Amplified DNA fragments were separated by electrophoresis 1.9 % agoras gel with 1xTAE-buffer (5). The 20 μl of volume was loaded on the gel. Gels were stained with ethidium bromide and fragment patterns were photographed.

Observations: Different fragments produced in each sample was scored and compared with each other (10).

Data analysis: Variability among species was expressed as the similarity "S". This is calculated as: S: 2 X N_{AB} / N_A + N_B in which N_{AB} are the number of bands shared by individuals A and B, N_A and N_B are the number of bands in individuals A and B, respectively. To be scored as present, the band had to be strong. The similarity measure can also be called band sharing. The

genetic distances can be calculated as D= 1-S. Common band analysis was conducted using a computer program developed in which makes a pairwise comparisons between all the species evaluated to determine the values of genetic distance. Dendograms were constructed from the genetic distance data by the SPSS computer program.

RESULTS AND DISCUSSION

It is becoming widely known that amplification results obtained with one RAPD primer on the same genotype can vary between laboratories, thermocyclers, source of polymerase, batches of reagents and DNA preparation (4). However, DNA fingerprinting of plant varieties is best achieved in vegetatively propageted species such as strawberries and potato and variabilty can be minimized. RAPD primers were used to characterize *Allium* species. Common bands were scrored as present or absent and the data were used to calculate the values of genetic distance among five *Allium* species.

The conditions were optimised in the previous studies. For each primer used, a multiple band profile was produced comprising from 5 to 10 major bands plus a varying number of minor bands (Figure 1). Some ambiquities arose in scoring of minor bands. Certain amplified bands appeared to be common to several species while other were present in some species but absent in others. The results were given in Table 1.

The genetical distances within the individuals of *A. karamanoglu* were low (0.05). This result is expected since the genetic variation between individulas of the same species should be low as compared the individuals of the other species. The ranges of values obtained among species were between 0.00 - 0.33. The highest genetic distances was obtained between *A. myrianthum* and *A. ilgazanse* and *A. karamanoglu* (0.33)

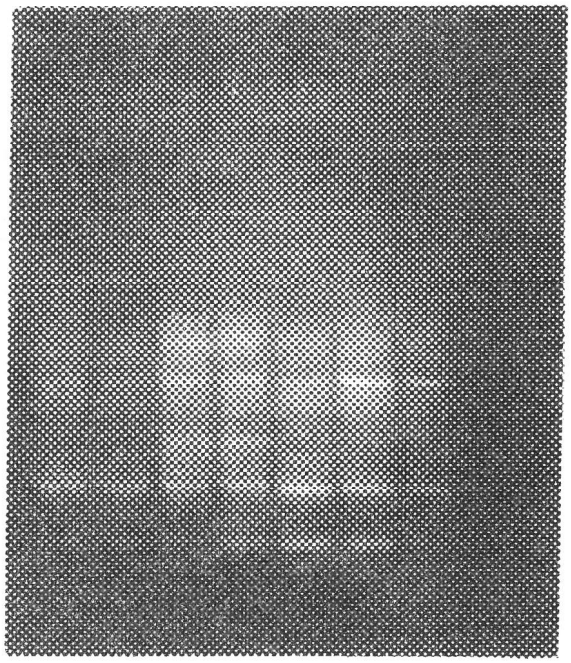

Figure 1. Polymorphic bands of amplified DNA produced in *A. myrianthum* (line 1), *A. isauricum* (line 2), *A ilgazasense* 1, *A ilgazasense* 2 (lines 3 and 4), *A. karamanoglu* 1, *A. karamanoglu* 2 (Lines 5 and 6) and *A. curtum* (Line 7 from left to right) with A1 primer.

Figure 1 shows that the results of an

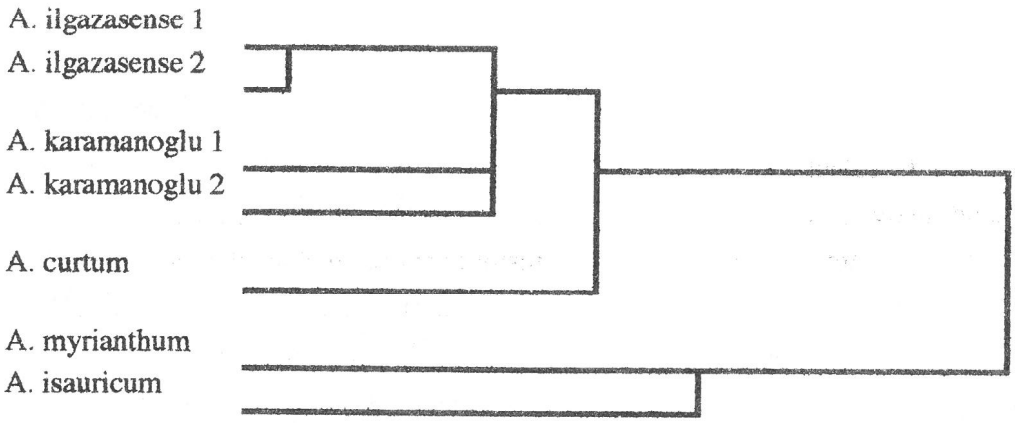

Figure 2. Dendogram generated by cluster analysis of genetic distance values given in Table 1 showing relations among different *Allium* species. Relative branch legths indicate relative genetic distance between species.

Table 1. Genetic distance values among *Allium* species calculated as described in Materials and Methods.

Species	*A.myri.*	*A.isau.*	*A.ılga -1*	*A.kara-1*	*A.kara-2*	*A.curt.*	*A. ılga-2*
A.myrianthum	0						
A.isauricum	0.0.9	0					
A.ılgazense-1	0.33	0.25	0				
A.karamanoğlu-1	0.33	0.25	0.10	0			
A.karamanoğlu-2	0.29	0.20	0.05	0.05	0		
A.curtum	0.23	0.14	0.11	0.11	0.06	0	
A. ilgazense-2	0.33	0.25	0.10	0.10	0.05	0.11	0

experiment in which single primers were used to amplify segments of genomic DNA from *A. isauricum, A. myrianthum, A. curtum, A. ılgazasense* and *A. karamanoglu.* At the intra specific level, two individuals from *A. ılgazansense* did not show polymorphism. Two individuals of *A. karamanoglu* also did not show polymorphism. However, comparison of different species of *Allium* was proved the polymorphism.

Cluster analysis of the genetic distance values conducted to generate dendograms indicating phylogenetic relations between *Allium* species studied (Figure 2). Cluster analysis is an standard method for analysing the relatedness of individuals from measured data (8). Dendograms generated using nearest neighbor, furthest neighbor and within group average, analysis were in general agreement with one another. A most closely related species were *A. ılgazasense* and *A. karamanoglu*, followed by *A. curtum, A. myrianthum* and *A. isauricum.* RAPD allowed to identify each species and the phlogenetic relations of *A. ılgazesense* and *A. karamanoglu* were agree the previous classification of Davis. Phylogenetic relations of *A. isauricum* and *A. curtum, A. myrianthum* were also agree with Davis's classification (2). However, comparison of these two group relations were found different than previous classifications. As a conclusion, we can say that the primer AI (operon) used in described conditions, is perfectly adapted to detection of these five *Allium* species.

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