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Determination of Genetic Polymorphism among Soybean (*Glycine max*. L. Merrill) Cultivars Developed in Recent Years through RAPD Markers

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Abstract: Genotype or cultivar identification is the first step to ensure plant breeder's rights. If registered cultivars or germplasms were not distinguished by any mechanism, then seed production or novel varieties could not be controlled. The RAPD markers are chosen to distinguish seven soybean (*Glycine max*. L. Merrill) cultivars from each other and to assign the polymorphisms among of them in this study. Eight primers were used and DNA fingerprinting of all cultivars were determined with that primers. According to that fingerprinting all cultivars were distinguished from each other in DNA banding pattern. Using these markers results, similarity index in between soybean cultivars were calculated. Dwight and Maverick were the most similar cultivars (These two cultivars were similar at 0,70 point to each other.) In addition that Maverick and Defiance cultivars have shown the highest polymorphism rate (These two cultivars were similar at 0,39 point each other).

***Keywords**:* (*Glycine max*. L. Merrill), soybean, RAPD, genetic polymorphism

1. Introduction

Soybean (*Glycine max.* L. Merrill), among cultivated products in the world, is one of the main sources of proteins and vegetable oils that are necessary for humans and animals. It is cultivated in many countries for having an important place in nutrition and industry due to its seeds rich in nutrients ratio. Its homeland is East Asia (China and Manchuria) (Markly, 1950). Soybean farming in Turkey has started in 1947. Countries that cultivate the most soybean in the world are, respectively, USA, China, Russia, Brazil, Indonesia, Korea, Japan and Canada. In Europe Romania and Turkey cultivate soybean greatly.

The importance of soybean in terms of nutrition and fat output was not understood until the 1940s. After the 1940s, intensive researches began on soybean farming and genetics in America (Fehr, 1987). Despite soybean seed collection being quite extensive, genetic basis of soybean cultivars is limited. Limited seed number at variety remediation has adversely affected the genetic basis of the modern soybean cultivars. Some objectives were realized by using the molecular markers in soybean farming. In selection studies, these studies have been used to select the better genotype or to transfer a desired gene from wild varieties into cultivated ones (Gizlice *et al*., 1994).

Random Amplified Polymorphic DNA (RAPD), a marker variant based on Polymerase Chain Reaction (PCR) method which does not require prior DNA information was developed (Williams *et al*., 1990a). The demonstration of differences between cultivars is very important. The RAPD markers efficiency and importance in determining this difference is significant. In this regard there are two objectives targeted in this study. The first of these is determination of

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genetic polymorphism between seven designated soybean cultivars by the use of RAPD markers. The second is, distinguishing cultivars from each other at molecular level with RAPD markers.

2. Materials and Methods

Material

In this study, seven soybean (*Glycine max.* L. Merrill) cultivars with American origins have been used as plant material. The seed introduction center in U.S. has been applied by Aegean Agricultural Research Institute in accordance with international agreements regarding exchange of plant genetic resources and Dwight, Maverick, Olympus, Savoy, Flint, Stride, Defiance cultivars have been provided. Soybean plant is divided in two maturation groups according to regions it is cultivated in the USA: First group comprises North American Cultivars; and second group South American Cultivars. Cultivars contained in these groups are given group numbers from 00, up to IV. Accordingly, Cultivars between 00 and III are called North American Cultivars; cultivars between III and IV, South American Cultivars. Group III is the transition group. First group also includes South Canadian Cultivars. Soybean cultivars chosen for the study are included in first group (North American).

DNA Isolation

Method proposed by Dellaporta *et al*. (1983) was applied at the genomic DNA isolation carried out with leaf samples taken from plants obtained from seeds. Quantification of isolated DNA and proper dilution ratios for DNA amount to be used in the PCR are determined by measurements made by UV spectrophotometer (Milton Roy 601). Genomic DNA samples were reserved at -20°C during laboratory studies.

PCR Phase

Eight different primers (Iontek) of 10 nucleotides in length were used in the study (Table 1). Main stocks were reserved at -20°C. Nucleotide sequences of the primers has been selected randomly, Guanine-Cytosine (GC) content between 50-80 % were preferred.

Table 1. Primers used at RAPD Analysis

Primer	Sequence
CS-44	5' ATTCGGCCG C 3'
CS-46	5' GGGATCTAG C 3'
CS-56	5' TGGTGGGTC C 3'
OH-04	5' GGAAGTCGC C 3'
OR-12	5' ACAGGTGCG T 3'
OS-03	5' CAGAGGTCC C 3'
SA-F	5' CGGCCCCTG T 3'
SA-R	5' AGGTCACTG A 3'

10X PCR buffer solution (100 mM Tris - HCL, 500mM KCL, pH 8,8 25°C), 1,5-2,0-2,5-3,0 mM MgCL2, 5 µM dNTPmix, 2 µM primer, 25 ng genomic DNA, 2,5 units Taq Polymerase enzyme (5U/pl) was added to PCR mixture and finally, to reach the 25 µl reaction volume, ultra pur distilled water was added. The DNA in the PCR mixture prepared in this manner, after being subjected to 94°C 5 min denaturation at Peltier Thermal Cycler PTC-200 PCR machine, was replicated for 94°C 1 min, 34°C 1 min, 72°C 3 min totaling 42 cycles, last elongation step of 72°C 10 min was performed and finally DNA was stabilized with 4°C 5 min application.

1% agarose gel (Sigma Agarose) was used for monitoring of the obtained PCR products. The photographs of the bands observed at the gel developed at 39V 40mM for 4 hours 20 mins, under UV light were taken using Foto Cap Machine.

Assesment of the Results

In order to calculate the similarity rate between two cultivars used in the committed study, the formula which was suggested by Sneath and Sokal (1973) was used.

Utilizing the gel photos the marker result charts were generated. The band patterns of varieties have been studied in the gel under UV light. It has been stated as "1" or "0" depending on whether there are bands or not. Non-homologous and homologous bands which were compared between the individuals which were respectively compared in two varieties were counted separately for each primer by using these charts and the similarity rate between two cultivars were calculated for each primer by using the similarity formula.

After that these rates were added and got the arithmetic average, the resulted figure in this process is the figure that gives the similarity result. According to these rates the dendogram (Figure 1) were formed by using Rohlf's (1990) computer program called "Numerical taxonomy and multivariate analysis system" (Version 1.8).

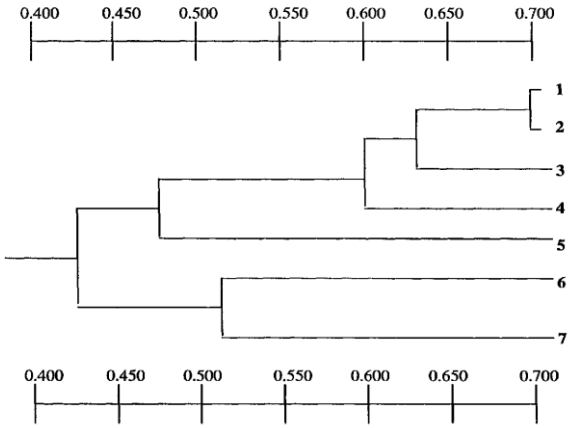

Figure 1. Dendogram which shows the similarity situation among the cultivars.
1.Dwight, 2.Maverick, 3.Olympus, 4.Savoy, 5.Flint, 6.Stride, 7.Defiance

3. Findings and Discussion

RAPD analysis revealed that with CS-44 primer 17 different markers, with CS-46 primer 13, with CS-56 primer 19, with OH-04 primer 22, with OS-03 primer 22, with OR-12 primer 13, with SA-F primer 20 and with SA-R primer 15 different markers were observed. These markers are heteromorphic. 141 markers were obtained as a result of this study. In electrophoresis analysis all of the eight primers got band. The obtained bands were found to beheteromorphic. This situation indicates that the arbitrary primers have complimentary series in template DNA which were isolated from the analyzed cultivars.

The similarity rates between the soybean cultivars studied were calculated by presuming that the similarity rate of the two cultivars which are 100 % same with each other is 1,00. According to the similarity rate values revealed that the Dwight and Maverick cultivars are two cultivars that has the highest similarity rate value with their value of 0,70. Following these two cultivars the Maverick-Olympus cultivars with the value of 0,64 and the Dwight-Olympus cultivars with the value of 0,63 have the secondly highest similarity rate values. The similarity rate value are gradually decreases and Maverick and Defiance have the lowest value of 0,39 and are two cultivates that have the lowest similarity rates. Table 2 which indicates the similarity rates can be seen below.

Table 2. Similarity Rates among Seven Soybean Cultivars

Cultivar	D.	M.	O.	S.	F.	S.	D.
Dwight	**1,00**						
Maverick	0,70	**1,00**					
Olympus	0,63	0,64	**1,00**				
Savoy	0,60	0,62	0,58	**1,00**			
Flint	0,44	0,45	0,50	0,54	**1,00**		
Stride	0,45	0,46	0,43	0,46	0,50	**1,00**	
Defiance	0,40	0,39	0,41	0,40	0,44	0,52	**1,00**

Using lesser DNA (15-25ng) in RAPD applications than in RFLP applications is sufficient. Besides that, there is no radioactive application within the processes. These markers are dominant. The RAPD technology shows the polymorphism placed on a very large amount of DNA locus successfully and quickly (Rafalski and Tingey, 1993).

Soy is a tetraploid plant with polyploid structure (Hymowitz and Singh, 1987) and consists of repeating the same genome. It acts diploid as cytogenetic, in other words its segmentation is like diploid structures. The comment in order to evaluate the similarity rate in two studies on wheat (Özden, 2000) and barley (Özüdoğru, 2000) that have similar analysis technique can not be apply to soybean. Because soy genome is approximately 1.1 x 109 bp (Arumuganathan and Earle, 1991). Through the genome structure and size of soy based on the RAPD markers that would be wrong to comment about its similarity rates.

Only if it works with co-dominant marker technique as RFLP or SSR it can be right to comment this way. Because of having dominant characteristics, the RAPD markers don't allow this kind of comment. Parental RFLP loci analysis showed that it has only two known alleles. However, due to the polyploid structure soybean RFLP probe is being hybridized and mapped more than one position in the genome typically. The main task of the polymorphic potential of RFLP probes to reveal the frequency of the two alleles at each locus probes. SSRs markers due to their multiple alleles of one locus polymorphic potential to reverse this condition depends on the number and frequency of alleles (Cregan *et al*., 1999).

In recent years, the development of new marker systems PCR-based AFLP, SSR, SNP, SRAP, will play a strategic role in molecular breeding studies managed in many plant species. The effort and cost spent per analysis will decrease with the development of technology and increase the chance of working with greater number individuals. As a result of these breeding process will be shortened, more accurate and detailed information obtained on the studied plants.

4. Conclusion

By using RAPD marker technique with eight randomly chosen primers, the DNA fingerprints of soybean cultivars were taken. As a result of the analysis it was determined that the soybean cultivars are highly resemble each other. When dendogram is observed, it can be seen that the similarity values vary between 0,70 and 0,39. Dwight-Maverick cultivars were the cultivars which have the highest rate of similarity with the value of 0,7. These two cultivars are the closest relative with each other as well. Maverick-Defiance cultivars are the cultivars which have the highest polymorphism and are the most distant relatives with the value of 0,39.

Consequently, the objectives targeted at the beginning of the study have been reached. In other words, the genetic polymorphism between RAPD markers and seven soybean cultivars have been presented and cultivars could be separated from each other at molecular level.

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