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Comparative Analysis of Agronomic Traits and ISSR Method among Some Soybeans [Glycine Max (L.) Merr.] Genotypes

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ABSTRACT
In this study, the genetic diversity was investigated among 12 soybeans genotypes using inter simple sequence repeats (ISSR) and agronomic traits. DNA was isolated from the leaves of the genotypes. For molecular characterization, a total of 26 primers of ISSRs and eight agronomic characteristics were evaluated. ISSR analysis revealed 88 polymorphic bands. The genetic diversity among the genotypes according to ISSR analysis and agronomic traits were estimated based on Nei homology and Euclidian distance, respectively, and dendrograms reflecting genetic similarity were constructed using UPGMA and NTSYSpc, respectively. Nei's homology coefficient values used for ISSR analysis ranged from 78%-84%, and the average Euclidean distance used for agronomic data ranged from 1.96-9.77. Although soybean genotypes evaluated in this study were highly similar, dendrograms showed that these genotypes could be distinguished both morphologically and genetically.

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Bazı Soya Fasulyesi [Glycine Max (L.) Merr.] Genotipleri Arasında Agronomik Özelliklerin ve ISSR Yönteminin Karşılaştırmalı Analizi

ÖZET
Bu çalışmada, 12 soya fasulyesi genotipi arasındaki genetik çeşitliliği, rastlantısal basit dizi tekrarları (ISSR) ve agronomik özellikler kullanarak araştırıldı. Bu genotiplerin yapraklarından DNA izole edildi. Moleküler karakterizasyon için, toplam 26 ISSR primeri ve sekiz agronomik özellik değerlendirildi. ISSR analizi 88 polimorfik bant ortaya çıkardı. ISSR analizine ve agronomik özelliklere göre genotipler arasındaki genetik çeşitlilik sırasıyla Nei homolojisi ve Euclidian mesafesine göre hesaplandı ve genetik benzerliği yansıtan dendrogramlar sırasıyla UPGMA ve NTSYSpc kullanılarak yapıldı. ISSR analizi için kullanılan Nei'nin homoloji katsayısı değerleri %78-84 arasında ve tarımsal veriler için kullanılan ortalama Euclidean mesafesi 1.96-9.77 arasında değişmiştir. Bu çalışmada değerlendirilen soya fasulyesi genotipleri oldukça benzer olmasına rağmen, dendrogramlar bu genotiplerin hem morfolojik hem de genetik olarak ayırt edilebileceğini göstermiştir.

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INTRODUCTION
Fabaceae is one of the largest families of plants and includes several economically important species (Ildis, 2001). The genus Glycine is one of the members of this family and is known showing distribution firstly in Asia and Australia (Baloch et al., 2010). Soybean (*Glycine max* L. Merr.) is believed to be originated and cultivated in China in the early 11th century (Fukuda, 1933; Yoon et al., 2009). USA is the largest producer of soybean in the world fallowed by China, Russia, Brazil, Indonesia, Korea, Japan, and Canada. In Europe, Romania, Yugoslavia and Turkey are also the largest producer of soybean (İşler and Çalışkan, 1998). It is believed that Caucasians introduced soybean into

Turkey from the Black Sea region after the First World War (Baloch et al., 2010).

Soybean is one of the world's most important grains crop because of its high protein and oil content (Lam et al., 2010; He et al., 2012). Approximately 30%-35% of the edible vegetable oil in the world is produced from soybean (Yılmaz and Efe, 1998). Additionally, seeds contain a high proportion of crude protein. This protein is one of easily digestible proteins. Soybean seeds are also rich in choline, pantothenic acid, niacin, thiamin, riboflavin, inositol, and vitamins A, B, and E. (Hassan, 2013). Therefore, the soybean sustains a global significance as important humans and animals food source (Singh et al., 2007).

Soybean yield in unit area varies based on varieties, cultivation practices, and ecological conditions (Agarwal et al., 2014). Crop yield is low especially in regions with a short growing season because of poor climatic conditions. Use of even transient species does not produce high yield in such regions. Early maturing genotypes are better adapted to changing environmental conditions. These genotypes flower at right time allowing higher crop yield (Yılmaz and Efe, 1998).

The identification of high yielding genotypes of crop plants is one of the most important tasks of plant breeders. Cultivars are identified on the basis of their morphological characteristics and are approved by The International Union for the Protection of New Varieties of Plants (UPOV). This approach is not ideal, as it is based on the identification of morphological features. Therefore, it is necessary to develop methods to quickly identify crop cultivars (Brick and Sivalop, 2001).

The investigation of morphological characteristics of crop plants are difficult because of limited number properties, low polymorphism, low heritability, late expression and in some cases the change of environmental factors (Agarwal et al., 2014). Therefore, morphological characteristics are inconclusive and cannot distinguish between closely related accessions or cultivars (Aghaei et al., 2012).

Knowledge of genetic diversity of cultivated plant species is highly important (Agarwal et al., 2014). Nowadays, DNA markers are widely used and are proven to be an efficient tool for the molecular characterization and determination of genetic diversity of crops (Meena et al., 2015).

Various PCR-based DNA markers, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), inter simple sequence repeat (ISSRs), amplified fragment length polymorphisms (AFLPs), single nucleotide polymorphisms (SNPs), and microsatellites or simple sequence repeats (SSRs) are used to determine the genetic diversity of plant species (Kumar 2009; Tantasawat et al., 2011; He et al., 2012).

ISSR markers are commonly used to analyze species diversity and to perform phylogenetic analysis, gene tagging, and genome mapping in various plant species (Brick and Sivolap, 2001; Baloch et al., 2010). ISSR markers are highly reproducible, as they use long primers (16-25 mers). Additionally, ISSRs usually contain a large number of polymorphic bands (up to 97) which allows detecting a high level of genomic polymorphisms (Monpara et al., 2017).

Main objective this study was to perform a comparative analysis of ISSR markers and agronomic traits for the selection of soybean genotypes.

MATERIAL and METHODS

Plant Material and DNA Extraction

Leaves of soybean genotypes used were obtained by the Konya Sarayönü Vocational School research land (Table 1). Genomic DNA was isolated from the leaf samples as described by Doyle and Doyle (1990) and quantified using NanoDrop ND-2000 Spectrophotometer (Thermo Fisher Scientific Inc., USA). The quality of DNA was analyzed on a 0.8 % agarose gel.

Polymerase Chain Reaction (PCR)

In this study, a total of 26 primers were tested for the PCR amplification of ISSR markers in the soybean genome (Table 2). PCR was performed in a 25 -µl volume containing 10X PCR reaction buffer, 3 mM $MgCl_2$, 2.5 mM dNTPs, 1.5 units Taq DNA polymerase (Fermentas, Thermo Fisher Scientific Inc., USA), 100 µM primer (Biomers, Germany), and 100 ng of template DNA on a Mastercycler Gradient thermal cycler (Eppendorf, North America), PCR conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 45 cycles of denaturation 94 °C for 1 min, annealing at 50 °C to 57 °C for 30 s, extension 72 °C for 40 s, and a final extension at 72 °C for 10 min.

The PCR products were separated by gel electrophoresis on 2% agarose gels. Subsequently, gels were stained with ethidium bromide and photographed on a UV transilluminator. Gel image was then transferred to the computer using DNA imaging system (Vilber Lourmat, Eberhardzell, Germany).

ISSR Data Analysis

Unambiguous ISSR bands app were visually scored for each primer as either present (1) or absent (0). Subsequently, scores of all primers were combined, and the genetic similarity was estimated based on Nei's homology using Bio1D++computer program (Vilber Lourmat, Bio1D++ Software) (Nei, 1978). Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA).

Table 1. Information about *Glycine max* (L.) Merr. Genotypes
Tablo 1. Glycine max (L.) Merr. Genotipleri hakkında bilgi

Genotype Genotip	Origin Köken	Variety or The Candidate Variety Improved Institution or Foundation Çeşit veya Aday Çeşit Geliştirildiği Kurum veya Kuruluş	Patently Variety Patentli Çeşitlilik	Maturity Group Olgunlaşma Grubu
A3935	America *Amerika*	Asgrow company *Asgrow Şirketi*	Variety *Çeşitlilik*	3.9
NE3399	America *Amerika*	Nebraska province *Nebraska ili*	Variety *Çeşitlilik*	3.9
DEFİENCE	America *Amerika*	America Ministry of Agriculture *Amerika Tarım Bakanlığı*	Variety *Çeşitlilik*	4
ARISOY	Turkey *Türkiye*	Çukurova University, Faculty of Agriculture *Çukurova Üniversitesi, Ziraat Fakültesi*	Variety *Çeşitlilik*	4
ATAKİŞİ	Turkey *Türkiye*	Çukurova University, Faculty of Agriculture *Çukurova Üniversitesi, Ziraat Fakültesi*	Variety *Çeşitlilik*	3.7
ATAEM 7	Turkey *Türkiye*	Western Mediterranean Agricultural Research Institute (BATEM) *Batı Akdeniz Tarımsal Araştırma Enstitüsü (BATEM)*	Variety *Çeşitlilik*	4.1
NOVA	Turkey *Türkiye*	Antalya; MAY Seed *Antalya; MAY Tohum*	Variety *Çeşitlilik*	3.8
BDS 27	Turkey *Türkiye*	Bahri Dağdaş International Agricultural Research Institute (BDUTEM) *Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü (BDUTEM)*	candidate variety *Aday çeşitlilik*	3.9
BDS 25	Turkey *Türkiye*	(BDUTEM) *	candidate varietiy *Aday çeşitlilik* candidate variety *Aday çeşitlilik*	3.8
BDS 21	Turkey Türkiye	(BDUTEM) *	candidate variety candidate variety Aday çeşitlilik	3.8
BDS 11	Turkey Türkiye	(BDUTEM) *	candidate variety candidate variety Aday çeşitlilik	3.9
BDS 07	Turkey Türkiye	(BDUTEM) *	candidate variety candidate variety Aday çeşitlilik	4

* candidate varieties having the same parents
* *aynı ebeveynlere sahip aday çeşitleri*

Evaluation of Agronomic Traits

Eight morphological features of soybean genotypes were evaluated. The average of three repeated measurements was statistically analyzed. According to the Euclidian distance, a dendrogram was obtained using the NTSYS-pcversion 2.1 statistical software package (Rohlf, 2000).

RESULTS

Evaluation of ISSR PCR Results

To identify the genetic diversity of soybean genotypes, 26 ISSR primers were tested, of which 25 successfully amplified genomic DNA, except for one primer (M4). The PCR products were evaluated based on electropherograms obtained with only four primer amplification (Figure 1).

Table 2. ISSR primers in which genetic variations of *Glycine max* (L.) Merr. genotypes are revealed
Tablo 2. *Glycine max* (L.) Merr. *genotiplerinin genetic varsyasyonunun ortaya konulduğu ISSR primerleri*

Primer code *Primer kodu*	Primer Sequence 5' →3' *Primer Dizisi 5' →3'*	Nucleotide Length *Nükleotit Uzunluğu*	Annealing temperature (°C) *Bağlanma Sıcaklığı* (°C)
UBC 808	AGAGAGAGAGAGAGAGC	17-mers	54
UBC 810	GAGAGAGAGAGAGAGAT	17-mers	52
UBC 812	GAGAGAGAGAGAGAGAA	17-mers	57
UBC 813	CTCTCTCTCTCTCTCTT	17-mers	52
UBC 825	ACACACACACACACACT	17-mers	53
UBC 827	TGTGTGTGTGTGTGTGA	17-mers	53
UBC 829	TATATATATATATATART	18-mers	52
UBC 840	GAGAGAGAGAGAGAGAYT	18-mers	54
UBC 841	GAGAGAGAGAGAGAGAYC	18-mers	54
UBC 843	CTCTCTCTCTCTCTCTRA	18-mers	52
UBC 848	CACACACACACACACARG	18-mers	54
UBC 850	GTGTGTGTGTGTGTGTYC	18-mers	56
UBC 852	TCTCTCTCTCTCTCTCRA	18-mers	52
UBC 855	ACACACACACACACACYT	18-mers	53
UBC 856	ACACACACACACACACYA	18-mers	54
M1	AGCAGCAGCAGCAGCAGCG	19-mers	56
M5	GAGAGAGAGAGAGAGAGAC	19-mers	56
M6	CACCACCACCACCAC	15-mers	50
M9	ACACACACACACACACCG	18-mers	52
M10	ACACACACACACACACC(C-T)	18-mers	54
M15	CACACACACACACACAAG	18-mers	50
M16	CACACACACACACACAGC	18-mers	54
M18	CGTCACACACACACACACA	19-mers	56
F1	AGAGAGAGAGAGAGAGTA	18-mers	52
UMN 001	CAGTGTGTGTGTGTGTGT	18-mers	50
M4 **	AGAGAGAGAGAGAGAG(C-T)C	18-mers	54

type of degenerate nucleotide: Y = pYrimidine (C, T); R = puRine (A, G). R = A-T, Y = G-C, B = T-G-C; D = A-T-G, H = A-TIC, V = A-G-C
dejenere nükleotid tipi: Y = pYrimidin (C, T); R=puRin (A, G). R = A-T, Y = G-C, B = T-G-C; D = A-T-G, H = A-TIC, V = A-G-C
**The primer didn't amplification
***Primer amplifikasyon yapmadı*

A total of 286 bands were obtained, of which ,88 were polymorphic, and 198 were monomorphic. The size of unambiguous ISSR bands varied from ~ 200 - 2000 bp. The lowest number of bands were obtained with UBC852 primer, and the highest number of bands were obtained with M1 primer. Primers UBC841 and UBC850 produced the smallest PCR fragment, and primers UBC852 and M10 produced the biggest PCR fragment.

The ISSR bands obtained from 12 soybean genotypes were evaluated using the UPGMA clustering analysis. Dendrogram constructed according to Nei's homology (Figure 2).

The 12 soybean genotypes formed two groups, each with 77% genetic similarity. Ten genotypes in similar rates of 78%-84% were included in the first group. Genotypes BDS11 and BDS21 were determined to be the most closely related, and BDS07 genotype was 82% similar to these genotypes. These three genotypes (BDS21, BDS11 and BDS07) had the same parents, which were the members of this group obtained from Bahri Dağdaş Agricultural Research Institute, and clustered in the third clade of the first main group. The genotype NOVA, which was an example of Antalya, (Turkey), also clustered in the same clade as BDS07, BDS11, and BDS21, with a genetic similarity of 79%. Four genotypes (BDS07, BDS11, BDS21, and BDS25), belonging to same parents, were clustered very close to each other; however, these genotypes could be distinguished from each other in the distance rate of 16%-22%. This observation is important in breeding studies carried to distinguish between genotypes. Other genotypes clustered in first main group were genetically very close to each other, with a genetic similarity of 78%-84%.

The second main group containing ATAEM7 and ATAKİŞİ genotypes showed only 23% genetic similarity to the first group, and both these genotypes showed 80% genetic similarity to each other. The genotype ARISOY was obtained from the Faculty of

Agriculture, Çukurova University, and the genotype A3935 was of American origin; both these genotypes in the first clade of the first main group with 81% genetic similarity. The genotype NE3399, also of American origin, clustered in same clade as ARISOY and A3935.

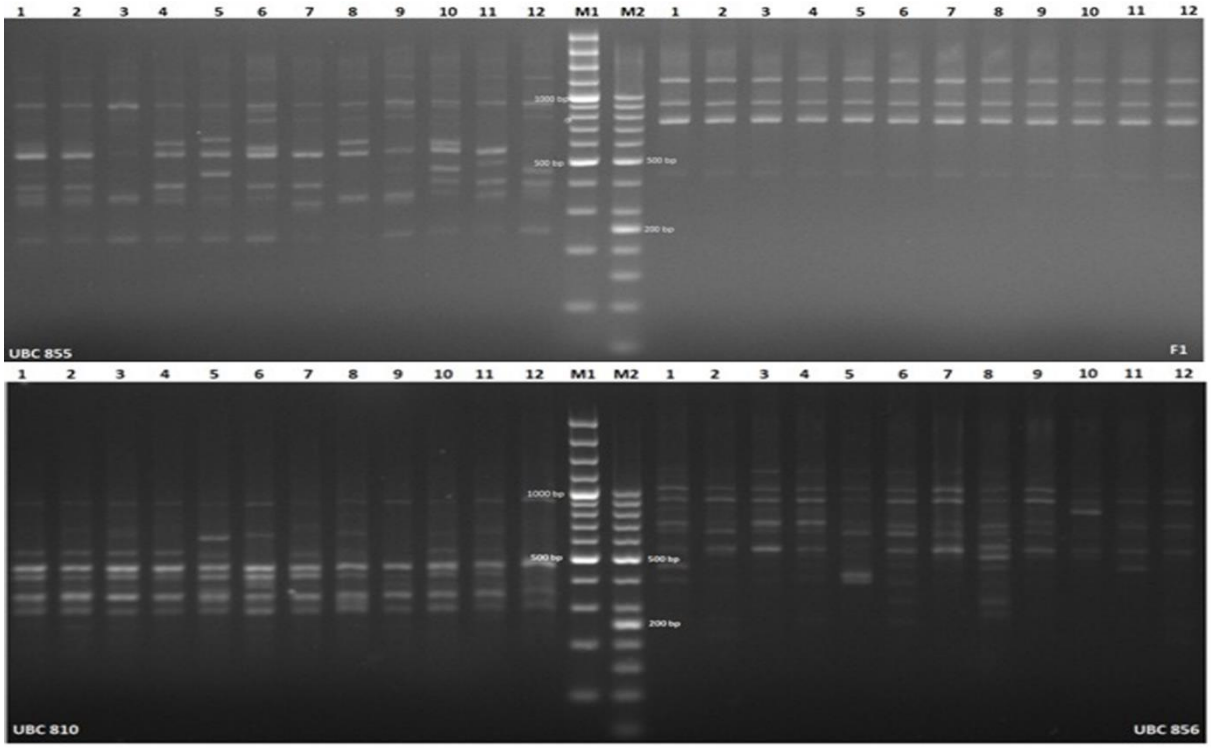

Figure 1. The agarose gel electrophoretogram of amplification products of DNAs obtained from different soybean genotypes. (Marker, M1: Fermentas SM1158; M2: Fermentas SM0371).

Resim 1. Farklı soya fasulyesi genotiplerinden elde edilen DNA'ların amplifikasyon ürünlerinin agaroz jel elektroforezi

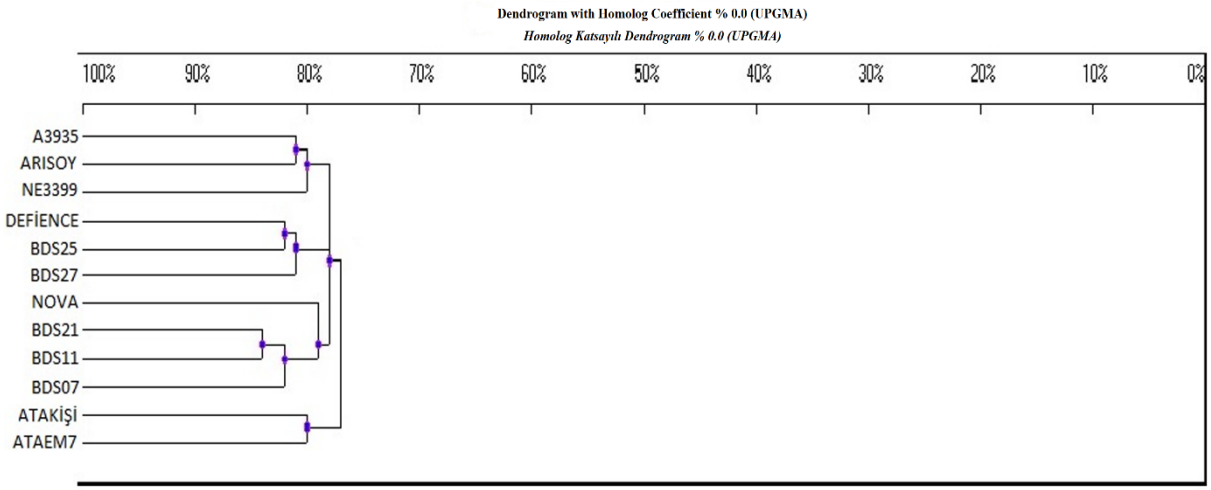

Figure 2. Dendrogram of genetic similarity obtained from ISSR-PCR profiles among Soybean genotypes

Resim 2. Soya fasulyesi genotipleri arasında ISSR-PCR profillerinden elde edilen genetik benzerlik dendrogramı

Evaluation of Agronomic Traits

Soybean genotypes were evaluated by eight agronomic characters that contribute to yield, including plant height, number of branches, number of pods, first pod height, pod length, number of seeds per pod, first branch height, and 100 grain weight (Table 3).

All of these eight agronomic characters are affected by environmental conditions, such as day length, more precise of photoperiod of late maturing genotypes according to early maturing genotypes and affected by day length differences of the number of pods (Whigham and Minor, 1978). The genotypes evaluated in this study were divided into maturity groups on the basis of day length; and the maturity groups ranged between 3.8 (BDS 21, BDS 25 and NOVA) and 4.1 (ATAEM 7).

Table 3. The numeric data of agronomic traits obtained from *Glycine max* (L.) Merr. Genotypes
Tablo 3. Glycine max (L.) Merr. Genotiplerinden elde edilen agronomik özelliklerin sayısal verileri

Genotypes *Genotipler*	Replication *Tekrar*	Plant height (cm) Bitki y*üksekliği (cm)*	Height to first branch (cm) *İlk dal yüksekliği (cm)*	Height to first pod (cm) *İlk kapsül yüksekliği* (cm)	Number of branch per plant *Bitki başına dal sayısı*	Number of pod per pant *Bitki başına kapsül sayısı*	Pod length (cm) *Tohum uzunluğu*	Number of seed per pod *Kapsül başına tohum sayısı*	100 seed weight (g) *100 tohum ağrlığı (g)*
DEFİANCE	1	51.20	5.20	12.10	2.40	22.20	4.00	2.80	12.65
	2	84.40	4.60	12.20	1.60	35.00	4.10	3.00	13.15
	3	70.60	6.80	17.20	1.70	22.30	3.70	2.90	11.81
BDS 07	1	111.60	7.50	24.30	2.00	30.80	4.00	3.00	12.51
	2	82.80	10.80	23.90	1.80	23.30	4.10	2.80	14.12
	3	74.80	10.10	18.20	2.00	20.50	3.30	3.00	11.60
BDS 27	1	76.50	7.60	14.90	3.40	29.40	4.14	3.00	13.85
	2	99.70	10.20	23.80	1.00	23.50	4.00	3.00	13.75
	3	77.30	9.30	26.30	1.50	22.50	3.70	3.00	11.55
NOVA	1	97.00	6.60	20.30	2.80	24.70	3.50	2.90	11.40
	2	84.30	6.20	17.00	1.90	33.20	3.60	2.70	11.32
	3	84.90	12.90	20.40	2.50	31.00	3.80	3.00	10.37
BDS 25	1	107.10	8.50	24.90	3.60	37.00	3.60	3.00	14.86
	2	92.60	8.10	24.50	3.70	33.30	4.00	3.00	14.07
	3	93.80	10.10	26.50	2.10	24.20	3.80	2.80	13.18
ATAEM 7	1	105.40	8.40	19.60	1.30	31.20	3.50	3.00	10.76
	2	93.80	1.80	17.70	0.20	20.80	3.80	2.70	10.72
	3	83.20	6.00	21.40	0.90	29.30	3.20	2.90	9.67
BDS 21	1	83.50	9.80	23.10	3.80	27.00	3.60	3.00	12.64
	2	75.20	8.80	22.40	3.60	27.10	3.40	2.60	12.04
	3	77.60	15.00	24.90	1.80	30.20	3.60	3.00	11.44
NE 3399	1	72.50	7.50	18.90	2.30	22.90	7.50	3.00	12.33
	2	92.20	9.90	20.30	2.00	32.50	3.70	2.50	12.91
	3	77.50	6.20	22.80	1.10	20.20	3.40	2.90	10.97
ARISOY	1	64.30	9.75	12.90	2.30	18.90	4.02	3.00	13.06
	2	106.70	7.20	25.50	4.00	32.50	4.00	3.00	10.69
	3	96.10	9.70	27.70	1.10	22.90	4.10	3.00	10.00
BDS 11	1	99.60	7.80	21.00	3.00	51.40	3.90	3.00	11.43
	2	102.40	6.40	20.40	1.80	40.60	4.00	3.00	11.72
	3	91.90	7.60	23.00	1.10	25.10	3.60	3.00	11.29
A 3935	1	73.30	6.10	15.00	4.30	45.10	4.10	3.00	12.71
	2	78.20	4.30	20.30	1.20	21.60	4.00	3.00	11.61
	3	77.70	7.00	32.40	2.40	28.10	3.40	2.80	11.10
ATAKİŞİ	1	82.30	6.10	13.80	4.70	51.50	4.10	3.00	11.54
	2	85.90	6.40	18.70	1.80	26.90	3.30	2.80	10.37
	3	87.40	8.20	23.40	3.10	30.90	3.80	2.60	9.85
F		N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
C.V. (%)		13	24	17	36	25	6	4	4
L.S.D. (0,05)			3.21						

A dendrogram of agronomic traits was calculated according to the euclidian distance (Figure 3). According to the dendrogram, the genotypes were grouped into two main groups. The first main group comprised two subgroups; the genotypes Defiance, A3935, NE3399, BDS21, BDS27 and, BDS07 clustered in the first subgroup, whereas ARISOY, ATAEM7, BDS11, NOVA and ATAKİŞİ clustered in the second subgroup. The genotype BDS25, which clustered in the second main group, was only 9.77% different from the first main group. Additionally, genotypes in the same maturity group clustered in different groups.

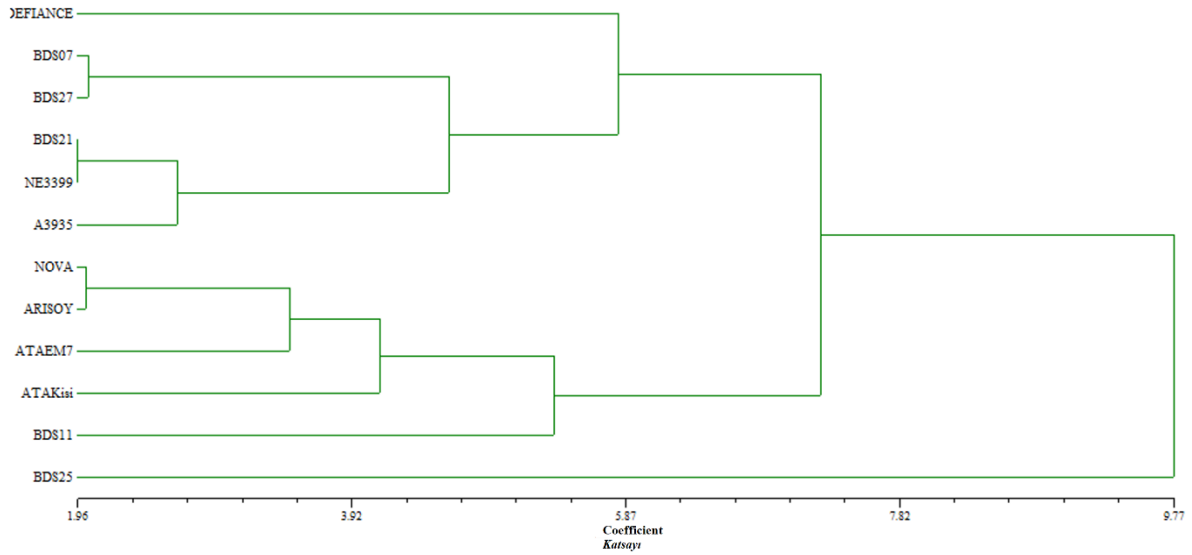

Figure 3. The dendrogram obtained according to the Euclidian distance based on the agronomic characters
Resim 3. Agronomik karakterlere dayanan Euclidian mesafesine göre elde edilen dendrogram

In the dendrogram obtained on the basis of agronomic traits, BDS21 and NE3399, NOVA and ARISOY and, BDS27 and BDS07 genotypes were the closest genotypes and candidate variety according to the morphological appearance. The genotype ARISOY and ATAKİŞİ, developed from the same ancestor, clustered in the second subgroup of first main group, with approximately 4% distance. Two genotypes (A3935 and NE3399), obtained from America, morphologically clustered in the same group. All of the sibling genotypes formed as a result of hybridization, to develop new varieties, did not show morphological similarity; instead these genotypes clustered in different groups.

Comparative Analysis of Dendrograms

We compared the dendrogram obtained using ISSR data from the agronomic traits. Soybean genotypes were much more closely related to each other according to the dendrogram obtained from agronomic traits than from that obtained from ISSR data. Genotypes from USA, including Defiance, NE3399 and A39035 clustered in the same group in both dendrograms. However, subgroups in the first main group showed differences between both dendrograms. Although genotypes were ~90% similar according to agronomic traits, candidate varieties clustered in different groups. Whereas, ISSR-PCR collected in a group, it achieved to distinguish with the distance rate of 16%-22%. Such data is useful in the development of varieties and selection of genotypes.

When analyzed in terms of morphological characteristics, the geographic distribution of genotypes, derived from the same parents clustered in different groups, despite being collected from the same place. This information could be related to phenotypic characteristics affected by different environmental conditions. If more morphological cha racteristics were examined, it could be expected that the overlap between dendrograms would be greater.

DISCUSSION

Molecular markers are advantageous for the identification of different genotypes and, have been utilized in population genetics studies (Abdelmigid, 2012; Chauhan et al., 2015). DNA based molecular markers have been invaluable in genetics and plant breeding (Nadeem et al., 2018). Among DNA markers, ISSRs are advantageous given the high annealing temperature of primers and high repetition, low cost and the genomic information provided. ISSRs have been widely used in plants, as they provide important information for understanding the relationship between species. Therefore, the results of this study may further assist in developing new breeding strategies (Abdelmigid, 2012). In this study, ISSR primers a total of 286 bands, with an average of 11.92 bands per primer were evaluated. The average

numbers of bands reported previously are 3.1 (Xie and Yoshihito, 2005), 20.05 (Arslan and Tamkoç, 2011), 5.47 (Bhatia et al., 2009), 18.8 (Abdelmigid, 2012), 6.5 (Aghaei et al., 2012) and 4.4 (Youssef, 2010).

Although our results demonstrated low genetic diversity among soybean genotypes using ISSR primers, these genotypes were successfully distinguished. Based on the number of polymorphic bands obtained in this study, DNA polymorphism was estimated as 30.76%. Low genetic diversity has also been reported previously using different DNA based molecular markers. For instance, AFLP markers have revealed 2.78% polymorphism among peanut genotypes (Herselman, 2003). In another study, the genetic diversity among 26 accessions of cultivated peanut has been determined as 18% by Dwivedi et al. (2001). RAPD markers have shown very low polymorphism among 18 soybean genotypes (Doldi et al., 1997). Ude and colleagues have reported 27% genetic diversity among 190 Japanese, North American, and Chinese soybean cultivars using AFLP markers (Ude et al., 2003).

Contrary to these results, Agarwal et al. (2014) have reported 77.89% polymorphism using 15 ISSR markers in soybean [*G. max* L. Merr.] genotypes. Jin et al. (2003) have shown high genetic diversity among 100 accessions of wild soybean (*Glycine soja* Sieb. and zucc.) collected from natural populations using 15 ISSR primers. Similarly, 60 SSR markers have revealed high genetic diversity among 123 soybean genotypes (Wang et al., 2006). Characterization of reproductive cell lines of pea (*Pisum sativum*) using RAPDs has shown genetic similarity index ranging from 26% to 79.3%; in this study 11 of 12 primers amplified the genomic DNA producing a total of 133 banding patterns (Yadav et al., 2007). Moreover, Brick and Sivolap (2001) have shown 75% polymorphism using ISSRs among 19 cultivars obtained from different ecological and geographic locations. Satyavathi et al., (2006) have demonstrated 95% genetic diversity among 72 soybeans cultivars collected from India using AFLP markers.

The information on genetic similarity among genotypes and populations is beneficial for breeding programs, as it ensures more powerful sampling of genotypes to be used for crossing for the development of new cultivars, and allows the organization of germplasm (Abdelmigid, 2012). In this investigation, the UPGMA analysis dendrogram and homology coefficient displayed strong relationship among soybean genotypes. The UPGMA analysis dendrograms generally did not show any clear clustering model according to where the accessions were collected (Abdelmigid, 2012). In our study, results of cluster analysis showed that were also placed in different cluster of geographically closer genotypes. Our results are consistent with those of Baloch et al. (2010) showing that ARISOY and A3935 genotypes clustered in the same group.

Today, both morphological and molecular approaches are used to distinguish between genotypes. One of the biggest challenges in soybean cultivation is the limited number of varieties. Soybean is much more sensitive to the environment, soil type, and day length than other plant species. Therefore, breeding for new and improved soybean varieties adapted to different environmental conditions is critical. The results obtained in this study showed that ISSR markers were able to distinguish between soybean genotypes. ISSRs are easier, cheaper, and more reliable than conventional breeding methods of morphological characterization, which are more laborious, costly, and time consuming. We believe that ISSRs will facilitate plant breeders in the development of high yielding varieties of crop plants.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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