

Assaying of diversity among soybean (*Glycin max* (L.) Merr.) and peanut (*Arachis hypogaea* L.) genotypes at DNA level

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Abstract: Developing successful strategies to ensure future increase in yield of soybean and peanut crops hinges in part, on improving the genetic basis of the cultivars. Knowledge of genetic relationships in crop breeding programs provides valuable information that can be used by plant breeders as a parental line selection tool. So far, a thorough analysis of genetic diversity among the soybean and peanut genotypes grown in Turkey had not been attempted at DNA level. In this study, inter-simple sequence repeats (ISSR) and sequence-related amplified polymorphism (SRAP) markers were used to evaluate the genetic diversity between 21 soybean and 32 peanut cultivars and breeding lines adapted to different regions of Turkey. The ISSR analysis, which was performed with 46 primers in soybean and 47 primers in peanut, yielded 31 and 26 polymorphic bands, respectively, while 26 and 17 polymorphic amplicons were amplified by 34 and 36 SRAP primer combinations in soybean and peanut, respectively. The unweighted pair group method with arithmetic means dendrograms (UPGMA), based on Jaccard similarities, were obtained from the combined ISSR + SRAP data for both soybean and peanut. In soybean, UPGMA dendrogram clustered all soybean cultivars into the same group except 'Yeşilsoy', whereas, in peanut, it separated cultivars southwest runner and spantex from all the other cultivars, breeding lines, and plant introductions. In light of the narrow germplasm base of soybean and peanut genotypes grown in Turkey, a renewed emphasis should be placed on the introduction of new sources of germplasm into the breeding pool in order to enhance genetic variability to permit continued progress in developing high yielding cultivars and lead to greater gains for selections. The results obtained from this study will be helpful for soybean and peanut breeders in Turkey to gain information about genetic diversity and will enable them to make a future strategy for broadening the genetic basis of these crops.

Key words: Genetic diversity, ISSR, SRAP, soybean, peanut, Turkey

Soya fasulyesi (*Glycin max* (L.) Merr.) ve yerfıstığı (*Arachis hypogaea* L.) genotipleri arasındaki çeşitliliğin DNA düzeyinde saptanması

Özet: Soya ve yerfıstığında verim artışını sağlamak için geliştirilecek başarılı bir ıslah stratejisi kısmen, çeşitlerin genetik temelini geliştirilmesine bağlıdır. Çeşit ıslah programındaki genetik yakınlığın bilinmesi ebeveyn seçecek bitki ıslahçıları için çok değerli bir bilgidir. Türkiye'de yetiştirilen soya ve yerfıstığının DNA düzeyindeki genetik çeşitliliği hakkında hiçbir bilgi bulunmamaktadır. Bu çalışmada, Türkiye'nin farklı bölgelerine adapte olmuş 21 soya ve 32 yerfıstığı çeşit ve hatlarındaki genetik çeşitliliği saptamak için ISSR ve SRAP markörleri kullanılmıştır. ISSR analizinde soyada 46, yerfıstığında 47 primer kullanılmış ve bunlarda sırasıyla 31 ve 26 polimorfik bant tespit edilmiştir. SRAP analizinde ise

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34 ve 36 primer kombinasyonu kullanılmış ve sırasıyla 26 ve 17 polimorfik ampikon elde edilmiştir. Soya fasulyesi ve yerfıstığı için kombine edilmiş ISSR ve SRAP verilerinden yararlanılarak elde edilen Jaccard benzerlik katsayılarına dayalı dendrogramlar oluşturulmuştur. Soyada UPGMA dendrogramında Yeşilsoy çeşidi hariç tüm çeşitler aynı grup içerisinde yer alırken, yerfıstığında Southwesterner ve Spantex çeşitleri diğer çeşitlerden ayrılmıştır. Türkiye'de yetiştirilen soya ve yerfıstığı çeşitlerindeki genetik çeşitliliğin çok düşük veya önemsiz olduğu görülmektedir. Halihazırda Türkiye'de yetiştirilen soya ve yerfıstığı çeşitlerinden farklı genetik temellere sahip gen kaynaklarının kullanılması genetik çeşitliliği artırabilir ve seleksiyonlarda daha büyük kazançlar sağlayabilir. Sonuç olarak bu çalışma Türkiye'deki soya ve yerfıstığı ıslahçıların genetik çeşitlilik hakkında bilgi edinmelerini sağlayacak ve bu bitkilerin genetik temellerini genişletmek için uygulayacakları stratejileri kolaylaştıracaktır.

Anahtar sözcükler: genetik çeşitlilik, ISSR, SRAP, soya, yerfıstığı, Türkiye

Introduction

Soybean (*Glycine max* (L.) Merr.) is a diploidized allotetraploid ($2n = 40$), autogamous plant classified in the legumes family Fabaceae. The genus *Glycine* includes about 20 annual and perennial species distributed primarily in Asia and Australia (Pearman 2005). It was cultivated in the eastern half of Northern China as early as the 11th century, but domestication probably occurred even earlier in the same region (Yoon et al. 2009). In the late 17th century, sailors brought soybean to Europe from China and Japan (Pearman 2005). In Turkey, it is assumed that soybean was probably first introduced after the First World War from the Caucasian to the Black Sea region (Arslanoğlu et al. 2005).

Cultivated peanut (*Arachis hypogaea* L.) is highly self pollinated, allotetraploid annual legume with $2n = 40$. There are 4 different market types of peanut, namely spanish, virginia, runner, and valencia, and the majority of varieties grown in Turkey are virginia type. Peanut is widely grown in more than 80 countries of America, Africa, and Asia (Pearman 2005). Peanut is native to South America, probably from a region including central Brazil and Paraguay. Spanish explorers took peanuts to Europe (Pearman 2005); however, our knowledge is limited as to how and when the peanut was introduced in Turkey, although it was first cultivated in the Thracian region (Arioğlu et al. 2003, Arioğlu 2007).

The history of soybean and peanut breeding in Turkey dates back 4 decades, with the goal of introducing plant material (PI) from abroad (Arioğlu et al. 2003, Arioğlu et al. 2005; Arioğlu 2007), and since then breeding efforts have gone several stages from adaptation, selection to commercialization. In the late 1980s and early 1990s, several soybean PI such

as Mitchell, A3127, and A3935, which were identified as the most suitable plant materials for different regions of Turkey, were registered and released as commercial cultivars (Arioğlu et al. 2005). Later on, various research stations particularly the Field Crops Department of Çukurova University and Çukurova Agricultural Research Institute in Adana, Turkey, concentrated on developing new soybean cultivars by using these plant introductions. These simple breeding efforts quickly produced soybean cultivars such as Umut 2002, Nazlıcan, and Türksoy in 2002 (Çeşit Kataloğu 2002).

Peanut breeding was initially started by Akdeniz Agricultural Research Station in Antalya by collecting local land races from different areas, and cultivars such as Çom and Gazipaşa were developed through the single plant selection method (Arioğlu 2007). Later peanut germplasm introduced from abroad was used for breeding some peanut varieties such as NC-7 and Florspan through the same institute (Çeşit Kataloğu 2008). To date, a few soybean and peanut cultivars have been released by national soybean and peanut breeding program with the contribution of different local agricultural stations and universities (Çeşit Kataloğu 2008).

Knowledge of genetic diversity and relationships among soybean and peanut genotypes commonly grown in Turkey may play a significant role in breeding programs to improve grain yield, oil and protein content and provide valuable information that can be used by plant breeders as a parental line selection tool. Different methods have been used to evaluate genetic diversity and pattern of variation in soybean and peanut such as pedigree analysis (Sneller 1994), assessing morphological traits (Oliveira and Valls 2003; Mebrahtu and Devine 2008), biochemical

markers (Narvel et al. 2000; Javed et al. 2004), and more recently DNA markers (Abe et al. 2003; Jin et al. 2006; Wang et al. 2006; Kamburona 2007; Feng et al. 2008).

ISSR (inter-simple sequence repeat) is a type of molecular marker, proposed by Zietkiewicz et al. (1994), for fingerprinting. The ISSR applies the principle of simple sequence repeat (SSR) and anchored polymerase chain reaction (PCR) amplification by designed primers that can randomly amplify DNA fragments between inversely inverted and closely spaced microsatellite, and the ISSR is simply inherited as dominant and reliable marker system for many organisms. Consequently ISSR fingerprinting has commonly been used in different crops such as lentil (Toklu et al. 2009), soybean (Brick and Sivolap 2001), peanut (Raina et al. 2001), pistachio (Kafkas et al. 2006), and chickpea (Bayraktar and Dolar 2009). Another useful marker system, Sequence-Related Amplified Polymorphism (SRAP), developed by Li and Quiros (2001), has been demonstrated to have several advantages over other techniques of DNA fingerprinting, as it is simple, easy to carry out, and could be more useful for routine germplasm screening. SRAP had successfully been applied in different areas of plant genomics such as genetic diversity and phylogenetic studies in different plants such as sugarcane (Suman et al. 2008), okra (Gülşen et al. 2007), alfalfa (Vandemark et al. 2006), and cucumber (Yeboah et al. 2008). However, in our literature survey, we did not find any published data describing the genetic diversity and the relationship of soybean and peanut genotypes using SRAP markers.

Several oil seed crops are grown and consumed in Turkey. Among oil seed crops, soybean and peanut have been earning popularity in the recent years, and used as food for human consumptions, animal rations and edible oils as well as in many industrial products. Besides being an important source of human food and animal feed, these crops also play an important role in maintenance of soil fertility by fixing nitrogen. In Turkey, soybean and peanut are cultivated on an area of 8,674.7 and 25,942.3 ha, with the production of 31,000 and 86,000 t, respectively (Turkish Statistical Institute 2008). In the last decade, the yield of soybean and peanut in Turkey has increased from 2270 and

2550 kg ha⁻¹ in 1998 to over 3540 and 3330 kg ha⁻¹ in 2008 (Turkish Statistical Institute 2008), largely due to improved agricultural and management practices. However, continued success to increase the yield of soybean and peanut in Turkey depends mainly on developing cultivars with high yield potential, resistance to various insects and pests, tolerance to different biotic and abiotic stresses, and having wide adaptation to different local environments, which in turn depends upon genetic variations.

So far, a thorough analysis of genetic diversity among the soybean and peanut genotypes grown in Turkey has not been attempted at the DNA level. Therefore, the objectives of the present investigation were (1) to assess genetic diversity by ISSR and SRAP marker between 21 soybean and 32 peanut genotypes (2) to determine the existence of unique (cultivars specific) and rare bands, and 3) to make a strategy for broadening the genetic base for future breeding of these crops.

Materials and methods

Plant material and DNA extraction

The details of 21 soybean and 32 peanut genotypes, which are grown in Turkey, are presented in Tables 1 and 2, respectively. Three seeds from each of the genotypes were grown in pots. Leaf samples were collected from each genotype as bulk. Genomic DNA was extracted from leaf tissue by the CTAB method of Doyle and Doyle (1990) with minor modifications of Özkan et al. (2005). The concentration of extracted DNA was estimated by comparing band intensity with λ DNA of known concentration, after 0.8% agarose gel electrophoresis and ethidium bromide staining. DNA was diluted to 5 ng μ L⁻¹ for ISSR and SRAP analysis.

ISSR and SRAP analysis

ISSR amplification was performed according to Zietkiewicz et al. (1994) with minor modification of Kafkas et al. (2006), using 46 ISSR primers [set no. 9, University of British Columbia (UBC), Vancouver, BC, Canada] for soybean and 47 for peanut. SRAP amplification was done according to Li and Quiros (2001), using 34 primer combinations for soybean and 36 primer combinations for peanut. The details

Table 1. Name, maturity group, date of registration and pedigree of 21 soybean cultivars, breeding line and plant introduction grown in Turkey.

Genotype	Maturity Group	Registration Date	Pedigree
Mitchell	IV	1986	Unknown
A3935	IV	1991	unknown
SA-88	III	1996	unknown
Türksoy	IV	2002	RHS6592/Mitchell 410
Umut-2002	III	2002	unknown
Nazlıcan	V	2002	RHS-6644/WARE
Nova	III	2005	Unknown
Arisoy	III	2006	S.4240/Williams
Atakişi	IV	2006	S.4240/ Williams
ATEM-7	IV	2006	unknown
Adasoy	IV	2009	S613129/Hrasoy
Yemsoy	IV	2009	RHS-6644/Hrasoy
Yeşilsoy	IV	2009	Dare/ JMS-2382
16-21	IV	Breeding line	Corsoy 79/ kent
36-37	III	Breeding line	William79/Corsoy 79
AW-4	III	Breeding line	A.3127/William
HA-11	III	Breeding line	William 79/S4240
HA-10A	III	Breeding line	William79/Corsoy 79
Amsoy71	II	Plant introduction	Unknown
Omaha	IV	Plant introduction	Unknown
S.4240	IV	Plant introduction	unknown

of primers of ISSR are illustrated in Table 3 and for SRAP in Table 4. Amplification reactions in both techniques were carried out in a 25 µL reaction mixture that contained 75 mM Tris-HCl, pH 8.8; 20 mM (NH₄)₂SO₄; 2.0 mM MgCl₂; 0.2 µM Primer; 100 µM each of dATP, dGTP, dCTP, and dTTP; 1 unit of *Taq* DNA polymerase; and 10 ng of genomic DNA.

All PCR amplifications were performed in a thermal cycler (Techne-412, Barloworld Scientific). Each PCR reaction was carried twice to check the reproducibility of bands. The ISSR included 1 cycle of 3 min at 940 °C, followed by 49 cycles of 1 min at 940 °C, 1 min at 400C to 600 °C (depending upon primer), and 2 min at 720 °C, followed by a final incubation for 7 min at 720 °C. In SRAP, the thermal cycler was programmed to 5 cycles of 1 min at 94 °C, 1 min at 35 °C, and 1 min at 72 °C, for denaturing, annealing, and

extension, respectively. Then the annealing temperature was raised to 50 °C for another 35 cycles. ISSR and SRAP amplification products were analyzed by gel electrophoresis in 2% agarose in 0.5× TBE buffer, stained with ethidium bromide, and photographed under ultraviolet light.

Band scoring and data analysis

The ISSR and SRAP bands were manually scored as present (1) or absent (0). Only clear and strong bands were recorded and used for analysis. Genetic similarities were calculated according to the method developed by Jaccard (1908). A Jaccard genetic similarity matrix was used to build an unweighted pair-group method with arithmetic means (UPGMA) tree. NTSYS-pc version 2.1 (Rohlf 2004) was used for genetic similarity computing and dendrogram construction.

Table 2. Name, registration date, market type and pedigree of 32 peanut cultivars and plant introduction grown in Turkey.

Genotype	Registration Date	Market Type	Pedigree
Çom	1986	Virginia	Selection from farmer Virginia stocks
Arioğlu-2003	2003	Virginia	PI 269084
Osmaniye-2005	2005	Virginia	75/1073 × NC7
Halisbey	2006	Virginia	75/1073 × NC7
Sultan	2006	Virginia	75/1073 × NC7
Çız-kırmızı	Breeding line	Virginia	75/1073 × NC7
Melez	Breeding line	Virginia	75/1073 × NC7
YF-13	Breeding line	Virginia	75/1073 × NC7
YF-17	Breeding line	Virginia	75/1073 × NC7
NC-7*	Plant Introduction	Virginia	NC5/F393,F334-3-5-51 (Florispán derived)/Jenkins Jumbo
Georgia Green*	Plant Introduction	Runner	Southern Runner/Sunbelt Runner
Southern runner*	Plant Introduction	Runner	PI203396/Florunner
Champs*	Plant Introduction	Virginia	VA 8911215/VA-C 92R
Brantley*	Plant Introduction	Virginia	unknown
Wilson*	Plant Introduction	Virginia	VA 781621/PI 47623
NC.V11*	Plant Introduction	Virginia	Florigant/NC5//Flogirant/PI 337396
Tampson-90*	Plant Introduction	Spanish	Selection in T × AG-5
Sunoleic-95R*	Plant Introduction	Runner	Sunrunner4 × F435-2-3-B-2-1-b4-B-3-b3-1-b
Southwest runner*	Plant Introduction	Runner	Comet × Florunner
Florida-MDR98*	Plant Introduction	Runner	Unknown
C-99R*	Plant Introduction	Runner	PI 203396/F427B-3-1-7-4,UF81206-1//72 × 32B-13-1-3, PI259785/Flogirant
ICGL-6*	Plant Introduction	Runner	unknown
Okrun*	Plant Introduction	Runner	Flurunner/Spánhoma
C76-16*	Plant Introduction	Spanish	unknown
Tamrun OL 01*	Plant Introduction	Runner	Tamrun 96/SunOleic 95R
Spantex*	Plant Introduction	Spanish	Selection from farmer Spanish stocks
Tifspan*	Plant Introduction	Spanish	Argentine(PI121070-1)/ PI405933
Starr*	Plant Introduction	Spanish	Spante/PI161317
Georgia runner*	Plant Introduction	Runner	Krinkle-leaf/PI331334
Georgia red*	Plant Introduction	Valencia	unknown
Georgia valencia*	Plant Introduction	Valencia	unknown
Spanco*	Plant Introduction	Spanish	Chico/Comet

*Pedigree of plant introduction material was taken from Isleib et al. (2001).

Table 3. The number of amplified and polymorphic bands produced by ISSR primers among 21 soybean and 32 peanut cultivars commonly grown in Turkey.

Primer Name	Soybean			Peanut			Amplified Bands	Polymorphic Bands	P %
	Sequence (5'-3')*	Annealing Temperature	Amplified Bands	Polymorphic Bands	P %				
UBC 807	(AG) ₈ T	50	5	0	0	4	1	25	
UBC 808	(AG) ₈ C	52	**	-	-	8	0	0	
UBC 809	(AG) ₈ G	52	7	0	0	7	0	0	
UBC 810	(GA) ₈ T	50	9	0	0	6	0	0	
UBC 811	(GA) ₈ C	52	4	0	0	5	1	20	
UBC 812	(GA) ₈ A	50	5	1	20	6	0	0	
UBC 813	(CT) ₈ T	50	9	0	0	-	-	-	
UBC 815	(CT) ₈ G	52	3	2	67	4	2	50	
UBC 816	(CA) ₈ T	50	-	-	-	4	0	0	
UBC 817	(CA) ₈ A	50	5	1	20	5	0	0	
UBC 818	(CA) ₈ G	52	3	0	0	7	3	43	
UBC 819	(GT) ₈ A	50	2	0	0	-	-	-	
UBC 820	(GT) ₈ C	52	-	-	-	2	0	0	
UBC 821	(GT) ₈ T	50	3	1	33	-	-	-	
UBC 822	(TC) ₈ A	50	7	3	43	-	-	-	
UBC 823	(TC) ₈ C	52	-	-	-	4	0	0	
UBC 824	(TC) ₈ G	52	6	0	0	2	1	50	
UBC 825	(AC) ₈ T	50	4	0	0	-	-	-	
UBC 826	(AC) ₈ G	52	6	2	33	3	1	33	
UBC 827	(TG) ₈ A	52	4	1	25	3	2	66	
UBC 828	(TG) ₈ C	50	4	1	25	-	-	-	
UBC 829	(TA) ₈ R T	52	4	0	0	2	1	50	
UBC 834	(AG) ₈ Y T	52	4	1	25	5	0	0	
UBC 835	(AG) ₈ YC	54	6	1	17	7	3	43	
UBC 836	(AG) ₈ YA	52	5	2	40	-	-	-	
UBC 840	(GA) ₈ YT	52	-	-	-	6	0	0	
UBC 841	(GA) ₈ YC	54	5	0	0	2	1	50	
UBC 842	(CT) ₈ YG	54	6	1	17	-	-	-	
UBC 843	(CT) ₈ RA	52	2	0	0	-	-	-	
UBC 844	(CT) ₈ RC	54	6	1	17	-	-	-	
UBC 845	(CT) ₈ RC	54	-	-	-	4	2	50	
UBC 847	(CA) ₈ RG	52	-	-	-	3	0	0	
UBC 848	(CA) ₈ RG	54	-	-	-	4	1	25	
UBC 850	(GT) ₈ YC	56	5	1	20	3	0	0	
UBC 851	(GT) ₈ YG	54	-	-	-	5	0	0	

Table 3. Continued.

Primer Name	Soybean		Peanut			Peanut		
	Sequence (5'-3')	Annealing Temperature	Amplified Bands	Polymorphic Bands	P %	Amplified Bands	Polymorphic Bands	P %
UBC 852	(TC) ₈ RA	52	-	-	-	1	0	0
UBC 855	(AC) ₈ YT	52	-	-	-	3	1	33
UBC 856	(AC) ₈ YA	52	-	-	-	3	1	33
UBC 857	(AC) ₈ YG	54	5	1	20	3	1	33
UBC 858	(TG) ₈ RT	52	-	-	-	4	1	25
UBC 859	(TG) ₈ RC	54	-	-	-	3	1	33
UBC 860	(TG) ₈ RG	52	3	0	0	3	1	33
UBC 861	(ACC) ₆	54	4	0	0	-	-	-
UBC 862	(AGC) ₆	56	8	0	0	-	-	-
UBC 864	(ATG) ₆	44	-	-	-	4	0	0
UBC 866	(CTC) ₆	56	4	0	0	-	-	-
UBC 867	(GGC) ₆	52	-	-	-	4	0	0
UBC 868	(GAA) ₆	48	8	4	50	5	0	0
UBC 869	(GTT) ₆	48	-	-	-	1	0	0
UBC 873	GAC AGA CAG ACA GAC A	48	5	1	20	4	0	0
UBC 874	CCC TCC CTC CCT CCC T	51	-	-	-	4	0	0
UBC 876	GAT AGA TAG ACA GAC A	48	-	-	-	6	0	0
UBC 878	GGA TGG ATG GAT GGA T	48	-	-	-	3	0	0
UBC 879	CTT CAC TTC ACT TCA	48	1	0	0	5	0	0
UBC 880	GGA GAG GAG AGG AGA	48	5	1	20	-	-	-
UBC 881	GGG TGGB GGT GGG GTG	60	-	-	-	4	1	25
UBC 887	DVD(TC) ₇	51	11	3	27	-	-	-
UBC 888	BDB(CA) ₇	51	6	1	17	-	-	-
UBC 890	VHV(GT) ₇	51	7	1	14	-	-	-
UBC 891	HVH(TG) ₇	51	6	0	0	-	-	-
Total/Average		202/5.18	31/0.79	15.34	171/4.07	26/0.61	15.2	

* R = A/T, Y = G/C, B = T/G/C; D = A/T/G, H = A/T/C, V = A/G/C

** Primer had not been applied in the respective crop.

Table 4. The forward and reverse primer sequences for SRAP analysis used in this study.

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
Me 1	TGAGTCCAAACCGGATA	Em 2	GACTGCGTACGAATTTGC
Me 2	TGAGTCCAAACCGGAGC	Em 3	GACTGCGTACGAATTGAC
Me 3	TGAGTCCAAACCGGAAT	Em 4	GACTGCGTACGAATTTGA
Me 4	TGAGTCCAAACCGGACC	Em 5	GACTGCGTACGAATTAAC
Me 5	TGAGTCCAAACCGGAAG	Em 6	GACTGCGTACGAATTGCA
Me6	TGAGTCCAAACCGGTAG	Em 7	GACTGCGTACGAATTATG
Me7	TGAGTCCAAACCGGTTG	Em 8	GACTGCGTACGAATTAGC
Me10	TGAGTCCAAACCGGGAC	Em 9	GACTGCGTACGAATTACG
Em 1	GACTGCGTACGAATTAAT	Em 10	GACTGCGTACGAATTTAG

Results

ISSR and SRAP analysis in soybean

Forty-six ISSR primer and 34 SRAP primer combinations were used to characterize 21 soybean genotypes. Out of the 46 ISSR primers, 6 (UBC830, UBC849, UBC865, UBC869, UBC875, and UBC877) did not give any amplification product (data not shown). Out of the remaining 40 ISSR primers, only 20 showed polymorphism and yielded 202 bands, of which only 31 bands were polymorphic (Table 3). The number of bands per primer ranged from 1 (UBC879) to 11 (UBC887), with an average of 5.18 bands per primer. The percentage of polymorphism ranged from 0% to 67% (UBC815), with an average of 15.3%. The average of Jaccard genetic similarity among soybean cultivars and advanced lines varied from 0.917 (Atakisi-Adasoy) to 0.994 (S4240-HA16/21, HA16/21-HA36/37), with an average of 0.959 (data not shown). In SRAP analysis, out of 34 primers, only 18 primer combinations gave polymorphic bands. Thirty-four SRAP primer combinations produced a total of 155 scorable bands, with an average of 4.66 bands per primer combination, of which 26 (17%) were polymorphic. The total number of amplified bands was between 2 (Me1Em9, Me2Em2, Me3Em2, Me3Em4, and Me7Em1) and 8 (Me1Em2 and Me2Em1); the number of polymorphic bands ranged from 0 to 3 (Table 5). The average of Jaccard genetic similarity among soybean cultivars and advanced lines varied from 0.911 (61/21-Yeşilsoy) to 1.000 (S4240-16/21), with an average of 0.959 (data not shown).

To obtain a more precise picture, we combined 2 data sets (202 ISSR and 155 SRAP bands) and measured genetic similarities among all cultivars and advanced lines. The genetic similarity among soybean cultivars and advanced lines varied from 0.922 (Atakışı-Adasoy) to 0.997 (S4240-16/21, 16/21-36/37), with an average of 0.959 (Table 6).

The general UPGMA dendrogram constructed using the combined data of the 2 sets of molecular markers (ISSR+ SRAP) is presented in Figure 1. The UPGMA dendrogram split the 21 soybean genotypes into 2 clusters, clusters A and B. Cluster A contained only 1 cultivar, Yeşilsoy whereas all other cultivars were grouped under the same cluster B. Cluster B was again divided into subgroup B1 containing 3 cultivars namely, Atakışı, HA-11, and HA-10A; the remaining cultivars were grouped under the same subcluster B2.

ISSR and SRAP analysis in peanut

In peanut, 5 out of the 47 ISSR primers did not produce any amplification products or profiles were not scorable. Twenty-three ISSR primers produced monomorphic patterns, whereas 14 primers produced only 1 polymorphic band. A total of 171 bands were amplified by 42 primers, out of which only 26 bands were polymorphic. The total number of amplified fragments was between 1 and 8, with an average of 4.07 bands per primer and the number of polymorphic bands ranged from 0 to 3, with an average of 0.61 bands per primer (Table 3). The average of Jaccard genetic similarity among peanut genotypes was 0.98, ranging from 0.92 to 1.0 (data not shown). In SRAP analysis, 4 primer combinations also

Table 5. The number of amplified and polymorphic bands produced by SRAP primers between 21 soybean and 32 peanut cultivars commonly grown in Turkey.

Soybean				Peanut			
Primer Combination	Amplified Bands	Polymorphic Bands	P (%)	Primer Combination	Amplified Bands	Polymorphic Bands	P (%)
Me1Em1	3	1	33	Me1Em2	6	0	0
Me1Em2	8	1	12.5	Me1Em3	6	1	16
Me1Em3	5	3	60	Me1Em4	5	1	20
Me1Em4	6	1	16	Me1Em5	8	0	0
Me1Em5	7	0	0	Me1Em6	9	0	0
Me1Em6	4	1	25	Me2Em1	7	0	0
Me1Em7	5	1	20	Me2Em4	6	0	0
Me1Em8	6	0	0	Me2Em5	8	0	0
Me1Em9	2	0	0	Me3Em8	7	0	0
Me1Em10	5	2	40	Me3Em10	5	3	60
Me2Em1	8	2	25	Me4Em8	5	0	0
Me2Em2	2	0	0	Me4Em9	7	1	14
Me2Em3	7	1	14	Me4Em10	2	0	0
Me2Em4	3	0	0	Me5Em1	6	1	16
Me3Em1	4	0	0	Me5Em2	9	0	0
Me3Em2	2	0	0	Me5Em3	5	0	0
Me3Em3	7	0	0	Me5Em4	2	0	0
Me3Em4	2	1	50	Me5Em5	8	1	12.5
Me4Em6	6	1	16	Me5Em6	9	0	0
Me4Em7	5	0	0	Me6Em1	5	2	40
Me5Em6	6	2	33	Me6 Em2	6	0	0
Me5Em8	7	2	28.5	Me6 Em3	5	1	20
Me5Em9	3	0	0	Me6 Em4	6	1	16
Me5Em10	5	0	0	Me6Em5	8	1	12.5
Me6Em6	3	0	0	Me6Em6	8	0	0
Me6Em8	3	1	33	Me8Em1	1	0	0
Me6Em10	3	1	33	Me8 Em3	2	0	0
Me7Em1	2	0	0	Me9 Em1	3	1	33
Me7Em2	3	0	0	Me9Em2	5	1	20
Me7Em3	3	1	33	Me9Em3	8	0	0
Me10Em3	3	0	0	Me10Em2	7	1	14
Me10Em5	5	1	20	Me10Em3	1	0	0
Me10Em6	5	0	0				
Me10Em6	5	0	0				
Me10Em10	7	3	43				
Total	155	26		Total	192	17	-
Average	4.56	0.76	16.77	Average	5.82	0.52	8.85

Table 6. The estimation of Jaccard genetic similarity through combined data of ISSR+ SRAP among 32 peanut genotypes grown in Turkey.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Arısoy	1																			
Atakişi	0.961	1																		
Nova	0.962	0.941	1																	
Türksoy	0.956	0.930	0.948	1																
Omaha	0.953	0.938	0.950	0.945	1															
SA-88	0.956	0.947	0.970	0.942	0.968	1														
S.4240	0.970	0.938	0.979	0.950	0.959	0.973	1													
Umut-2002	0.967	0.940	0.961	0.967	0.967	0.961	0.970	1												
AITEM-7	0.956	0.941	0.959	0.936	0.956	0.965	0.973	0.955	1											
A.3935	0.970	0.944	0.973	0.951	0.965	0.968	0.970	0.961	0.967	1										
16-21	0.968	0.936	0.976	0.954	0.956	0.971	0.997	0.973	0.971	0.968	1									
36-37	0.967	0.935	0.973	0.953	0.953	0.967	0.993	0.969	0.967	0.964	0.997	1								
AW-4	0.967	0.952	0.958	0.953	0.967	0.970	0.967	0.978	0.970	0.967	0.964	0.961	1							
HIA-11	0.961	0.969	0.964	0.935	0.943	0.964	0.967	0.946	0.964	0.967	0.964	0.960	0.952	1						
HIA-10A	0.951	0.958	0.937	0.948	0.951	0.948	0.951	0.953	0.937	0.945	0.948	0.944	0.953	0.958	1					
Nazlıcan	0.948	0.941	0.950	0.951	0.959	0.945	0.947	0.967	0.942	0.953	0.951	0.947	0.959	0.944	0.956	1				
Amsoy71	0.953	0.932	0.950	0.945	0.964	0.956	0.959	0.979	0.939	0.953	0.962	0.958	0.961	0.937	0.956	0.964	1			
Adasoy	0.942	0.922	0.928	0.934	0.965	0.945	0.936	0.944	0.939	0.954	0.934	0.930	0.945	0.932	0.951	0.937	0.953	1		
Yemsoy	0.959	0.944	0.956	0.962	0.970	0.962	0.959	0.990	0.956	0.965	0.962	0.958	0.979	0.949	0.956	0.970	0.970	0.948	1	
Mitchell	0.961	0.934	0.952	0.964	0.955	0.946	0.955	0.987	0.940	0.961	0.958	0.957	0.963	0.939	0.947	0.967	0.972	0.941	0.978	1
Yeşilsoy	0.958	0.931	0.937	0.949	0.935	0.938	0.940	0.954	0.938	0.952	0.943	0.940	0.955	0.942	0.932	0.943	0.938	0.938	0.958	0.948

The average genetic similarity, 0.959

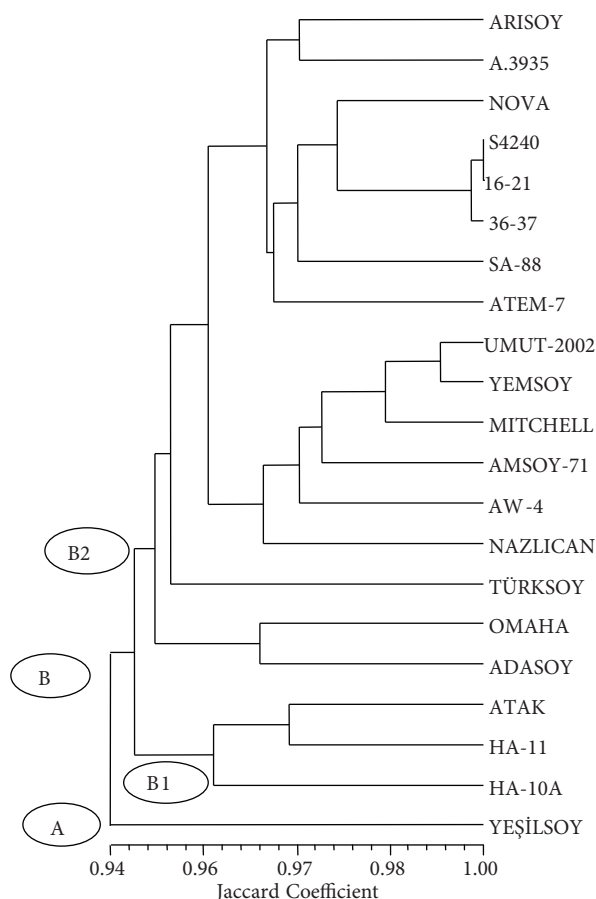


Figure 1. Association among 21 soybean genotypes grown in Turkey as revealed by UPGMA cluster analysis of Jaccard genetic similarity coefficient calculated from combined data of ISSR and SRAP.

did not produce any amplified products. The remaining 32 primers combinations produced a total of 192 bands with an average of 5.82 bands per primer combination. Out of the 32 amplified primers, 13 primers had polymorphic and 19 primers had monomorphic patterns. The total number of amplified bands ranged from 1 (Me8Em1, Me10Em3) to 9 (Me1Em6, Me5Em6, Me5Em2). The total number of polymorphic bands ranged from 0 to 2 with an average of 0.52 bands per primer combination (Table 5). The average Jaccard genetic relatedness was 0.975, ranging from 0.92 to 1.0 (data not shown).

The combined data of both marker systems (171 ISSR and 192 SRAP bands) were used to calculate genetic similarities to gain a clearer understanding about the genetic relatedness of peanut. Jaccard

genetic similarity ranged from 0.93 (6-20) to 1.00 (9-12, 11-29) with an average of 0.97 (Table 7).

The ISSR + SRAP (combined) based UPGMA dendrogram separated all 32 peanut genotypes into 2 main groups: A and B (Figure 2). Group A contained only 2 genotypes, namely Southwest runner and Spantex, whereas all other genotypes were placed under the same group, B.

Discussion

Despite the existence of substantial diversity among peanut and soybean genotypes for various morphological, agronomical, and physiological traits, very little DNA variation had been detected by using protein, isozymes, or DNA based markers by various previous researchers worldwide (Halward et al. 1991; 1992; Doldi et al. 1997; Dwivedi et al. 2001; Herselman 2003; He et al. 2003; Javed et al. 2004; Naito et al. 2008). Soybean and peanut breeding procedures with intensive selection pressure led to a dangerously narrowed genetic basis of the currently improved soybean and peanut varieties throughout the world.

In this study, ISSR primer and SRAP primer combinations resulted 16% and 22%, and 11% and 13% polymorphism in soybean and peanut, respectively (Tables 3 and 5). The sort of genetic variability in both crops we found is of the same type as reported by different previous researchers. For example, Herselman (2003) found a very low level of polymorphism (on average 2.78%) among 21 closely related cultivated South African peanut genotypes using AFLP marker, and Dwivedi et al. (2001) reported a low genetic diversity (18%) among 26 accessions of cultivated peanut, including interspecific derivatives, landraces, and released cultivars using RAPD markers. Halward et al. (1991, 1992) used a single primer DNA amplification for genetic studies among 25 genotypes of *Arachis hypogaea* and found no variation in banding patterns. He and Prakash (1997) reported narrow polymorphism (3%) in cultivated peanut by using a DAF (DNA amplification fingerprinting) approach, and Kochert et al. (1991) found a very low level of RFLP variability among 8 US peanut cultivars and 14 wild *Arachis* species. Similarly various researchers also reported a low

Table 7. The estimation of Jaccard genetic similarity through combined data of ISSR+ SRAP among 32 peanut genotypes grown in Turkey.

Genotypes	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
Halisbey	1.000														
Sultan	0.989														
Osmaniye2005	0.986	0.981													
Arroğlur-2003	0.962	0.962	0.970												
Çom	0.978	0.984	0.970	0.962											
NC-7	0.981	0.986	0.973	0.965	0.992										
Georgia Gren	0.981	0.986	0.973	0.965	0.986	0.989									
Southern Runner	0.970	0.981	0.978	0.969	0.975	0.978	0.978								
YF-13	0.983	0.989	0.975	0.967	0.989	0.991	0.991	0.980							
Champs	0.983	0.983	0.978	0.975	0.987	0.981	0.981	0.970	0.983						
Brantley	0.986	0.992	0.978	0.970	0.992	0.994	0.994	0.986	0.997	0.986					
YF-17	0.978	0.984	0.970	0.968	0.984	0.986	0.986	0.975	0.989	0.978	0.992				
Wilson	0.978	0.984	0.970	0.968	0.984	0.986	0.986	0.975	0.989	0.984	0.992	0.989			
NC.V.11	0.986	0.991	0.978	0.970	0.991	0.994	0.994	0.983	0.997	0.986	0.992	0.991	0.991		
Çiz kirmızı	0.981	0.986	0.978	0.970	0.986	0.989	0.989	0.978	0.991	0.981	0.994	0.986	0.991	0.994	
Melez	0.981	0.986	0.973	0.965	0.986	0.994	0.989	0.978	0.991	0.981	0.994	0.986	0.992	0.994	0.994
Tamspan	0.946	0.952	0.938	0.936	0.962	0.960	0.954	0.945	0.956	0.946	0.960	0.957	0.962	0.959	0.959
2-Sunoleic-95R	0.978	0.984	0.976	0.968	0.984	0.986	0.986	0.975	0.989	0.984	0.992	0.984	0.984	0.991	0.986
3-Southwest Runner	0.946	0.946	0.954	0.941	0.936	0.934	0.939	0.956	0.935	0.936	0.939	0.941	0.941	0.938	0.943
4-FloridaMDR-98	0.981	0.986	0.973	0.965	0.986	0.989	0.989	0.978	0.991	0.981	0.994	0.986	0.986	0.994	0.989
6-C-99-R	0.978	0.984	0.981	0.973	0.984	0.986	0.986	0.986	0.989	0.978	0.992	0.984	0.984	0.991	0.986
7-ICGL-6	0.958	0.958	0.945	0.942	0.968	0.965	0.960	0.952	0.962	0.958	0.965	0.958	0.963	0.965	0.965
Okrun	0.978	0.978	0.970	0.956	0.978	0.981	0.981	0.969	0.983	0.983	0.986	0.978	0.989	0.986	0.986
C76-16	0.981	0.986	0.972	0.964	0.986	0.989	0.989	0.977	0.991	0.983	0.994	0.986	0.994	0.994	0.994
Tamrun OL-01	0.984	0.984	0.970	0.968	0.984	0.986	0.986	0.975	0.989	0.984	0.992	0.984	0.991	0.991	0.986
Spantex	0.940	0.935	0.948	0.945	0.951	0.943	0.938	0.947	0.940	0.951	0.943	0.940	0.951	0.942	0.947
Tifspan	0.958	0.963	0.955	0.952	0.978	0.971	0.965	0.959	0.968	0.958	0.971	0.968	0.973	0.970	0.975
Starr	0.984	0.989	0.976	0.968	0.989	0.992	0.992	0.981	0.994	0.984	0.997	0.989	0.989	0.997	0.991
Georgia Runner	0.968	0.973	0.960	0.952	0.978	0.976	0.976	0.965	0.978	0.968	0.981	0.984	0.973	0.981	0.975
Georgia Red	0.971	0.976	0.963	0.955	0.976	0.979	0.978	0.967	0.981	0.971	0.984	0.976	0.981	0.983	0.983
Georgia Valencia	0.984	0.989	0.976	0.968	0.989	0.992	0.992	0.981	0.994	0.984	0.997	0.989	0.989	0.997	0.991
Spanco	0.973	0.978	0.965	0.952	0.978	0.976	0.976	0.967	0.978	0.968	0.981	0.973	0.973	0.981	0.975
Tamspan	0.960	0.957													
2-Sunoleic-95R	0.986	0.930													
3-Southwest Runner	0.939	0.960	0.941												
4-FloridaMDR-98	0.989	0.986	0.986	0.939											
6-C-99-R	0.986	0.957	0.989	0.946	0.986										
7-ICGL-6	0.965	0.973	0.963	0.936	0.966										
Okrun	0.986	0.957	0.983	0.935	0.981	0.963									
C76-16	0.994	0.964	0.986	0.938	0.989	0.986	0.962								
Tamrun OL-01	0.986	0.952	0.984	0.936	0.986	0.984	0.973	0.989							
Spantex	0.943	0.945	0.946	0.943	0.951	0.962	0.973	0.986	0.951						
Tifspan	0.971	0.973	0.968	0.971	0.968	0.978	0.978	0.978	0.967	0.967					
Starr	0.992	0.962	0.994	0.992	0.994	0.968	0.989	0.991	0.989	0.946	0.973	0.984			
Georgia Runner	0.976	0.962	0.979	0.936	0.978	0.968	0.973	0.981	0.973	0.946	0.973	0.984	0.981		
Georgia Red	0.984	0.970	0.981	0.944	0.984	0.981	0.976	0.981	0.989	0.954	0.981	0.986	0.981		
Georgia Valencia	0.992	0.962	0.994	0.941	0.992	0.994	0.989	0.991	0.989	0.946	0.973	0.100	0.984	0.986	
Spanco	0.976	0.968	0.979	0.941	0.978	0.973	0.973	0.981	0.973	0.946	0.973	0.984	0.989	0.981	0.984

The average genetic similarity: 0.97

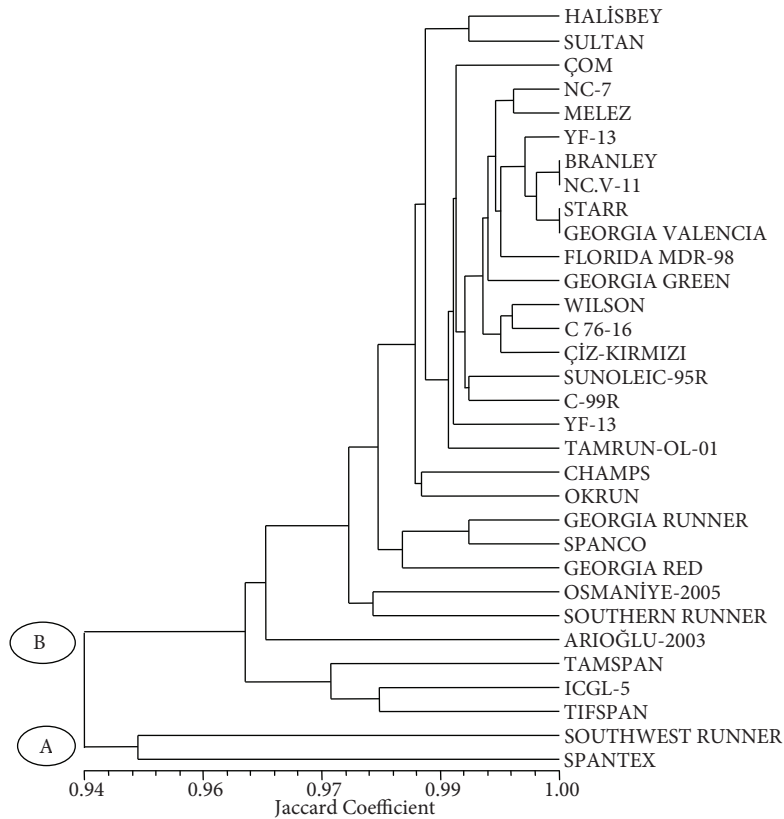


Figure 2. Association among 32 peanut genotypes grown in Turkey as revealed by UPGMA cluster analysis of Jaccard genetic similarity coefficient calculated from combined data of ISSR and SRAP.

genetic diversity in cultivated soybean. For instance, Doldi et al. (1997) found a low level of polymorphism among 18 soybean genotypes. Moreover, Ude et al. (2003) reported a low genetic diversity (27%) among 190 Chinese, Japanese, and North American soybean cultivars by using AFLP primers. The lack of detectable variation within soybean and peanut genotypes is in contradiction to the findings of various earlier studies. Brick and Sivalop (2001), who used 5 selected ISSR primers to study 19 cultivars of soybean of different ecological and geographic origins, detected 75% polymorphism. Wang et al. (2006) observed a high level of genetic diversity among 123 accession of cultivated soybean from China using 60 SSR markers. By using AFLP markers, Satyavathi et al. (2006) reported 95% polymorphism among 72 soybean varieties under cultivation in India. Raina et al. (2001) used 29 ISSR primers to assess genetic variation and the interrelationship

between subspecies and botanical varieties of cultivated peanut and wild species of *Arachis hypogaea* and detected 54.4% polymorphism. Mondal et al. (2008) observed 74.5% polymorphism among 19 peanut cultivars and breeding lines and wild species by using 21 selected ISSR primers. High level of DNA polymorphism in the above reported studies might also be attributed to the use of wild and different botanical species, and also to the use of different selected primers, making the results not easily comparable.

Molecular markers have provided a powerful new tool for breeders to search for new sources of variation and to investigate genetic factors controlling quantitatively inherited traits (Dwivedi et al. 2001; Satyavathi et al. 2006; Altıntaş et al. 2008). Various molecular markers showed different efficiency for evaluating DNA fingerprinting in soybean and peanut. For example, Narvel et al. (2000) used 74 SSR

to analyze 39 elite soybean genotypes from North America and found 3.5 polymorphic bands per primer pair. Li and Nelson (2001) used 35 random amplified polymorphic DNA (RAPD) for 120 soybean accessions from China, Korea, and Japan and observed 3.3 polymorphic bands per primer. Ude et al. (2003) used 5 amplified fragments length polymorphism (AFLP) primer pairs to study genetic diversity among 35 North American soybean ancestors, 66 high yielding North American soybean cultivars, 59 modern Chinese cultivars, and 30 modern Japanese cultivars and observed 18 polymorphic fragments per primer pair. In peanut, Naito et al. (2008) used 13 SSR primers to analyze 201 accession of *A. hypogaea* and 13 accessions of *Arachis* wild species and detected 108 polymorphic alleles in *A. hypogaea* with an average of 8.3 bands per SSR. Mondal and Badigannavar (2009) used 26 selected polymorphic SSR primers to characterize molecularly 20 cultivated peanut genotypes and found 104 polymorphic alleles with an average of 4 alleles per SSR. In this study, we used 2 different molecular markers, ISSR and SRAP, to estimate genetic diversity in soybean and peanut; we observed 202 and 155 bands, 171 and 192 with an average of 0.79 and 0.61, 0.76 and 0.52 polymorphic loci per primer/primer pair in soybean and peanut, respectively. We used 2 different marker systems to obtain a clearer picture about genetic variations of soybean and peanut genotypes grown in Turkey; however, neither of these markers found DNA polymorphisms in soybean and peanut genotypes grown in Turkey. Therefore, further study is needed using different DNA markers, which might be able to detect genetic diversity between cultivated soybean and peanut genotypes; however, soybean and peanut cultivars grown in Turkey are highly genetically similar.

The band present in only one cultivar is termed a unique or cultivar specific band. In the present study, we found 4 unique bands in 4 soybean cultivars (Nazlıcan, Atakişi, 36/37, and Mitchell). The position at which bands were present in only 2 out of 21 cultivars was termed as rare bands. There were also such 3 bands positions distributed in 4 soybean cultivars, namely Adasoy, Türksoy, Mitchell, and Yeşilsoy. Unique bands were amplified by 2 ISSR primers (UBC821 and UBC868) and 2 SRAP primers

(Me1Em4 and Me10Em10), whereas rare fragments were produced by 2 ISSR primers (UBC822, UBC826) and 1 SRAP primer combination (Me6Em10). In peanut, 5 cultivars, namely YF-13, Sunoleic-95R, Florida-MDR98, Spantex, and Georgia Valencia, have unique bands. Cultivar YF-13 had 2 unique bands. Unique bands were produced by 4 ISSR primers (UBC818, UBC826, UBC824, and UBC891). ISSR primer UBC818 amplified 3 cultivar specific bands distributed between 2 cultivars (cultivar YF-13 produced 2 unique bands and cultivar Sunoleic 95R had 1 specific band). Rare bands were amplified by 2 ISSR primers (UBC811 and UBC 835) and 1 SRAP primer combination (Me3Em10). Rare bands were distributed over 5 peanut cultivars, namely NC-7, Melez, Tampsan, ICGL-6, and Tifspan. If these bands are confirmed over a broader range of accessions, then these cultivar specific unique bands can be useful for the identification of these 4 cultivars directly or after the development of sequence-tagged site primers.

Information deduced from the dendrogram showed a very narrow genetic basis of soybean and peanut. Based on the combined ISSR and SRAP data, all soybean cultivars and advanced line, except Yeşilsoy, were grouped together under the same cluster, which is in accordance with the pedigree information (Table 1). Most of the soybean cultivars were developed by using a limited number of parents (Table 1). For example, genotype 16-21 and 36-37 constitute a small subgroup and are similar to each other; both originated a cross involving 1 common parent, corsoy 79; similarly, 6 soybean cultivars, AW-4, Arısoy, Atakişi and HA-11, HA-10A, and 36-37, which were released from Çukurova University, were developed from the crosses having Williams as one of the parents. Similarly, based on the combined ISSR and SRAP data, grouping of peanut genotypes in UPGMA dendrogram was also according to pedigree histories. Peanut cultivars and breeding lines such as Melez, Halisbey, Sultan, YF-13, YF-17, Çız-kırmızı, and Osmaniye-2005 were developed through the same cross combinations 75/1073 × NC7. Our results revealed that the genetic bases of released cultivars of soybean and peanut in Turkey are extremely narrow and there is urgent need to broaden the genetic basis.

Our results have relevant implications for Turkish soybean and peanut breeding. Soybean and peanut breeding materials and cultivars grown in Turkey are direct introduction from abroad, particularly the USA. Soybean and peanut cultivars grown in Turkey originated from intraspecific crosses of few local adapted cultivars or germplasm, particularly with superior agronomic characters. These crossing experiments involve the use of related parents in breeding programs; as a result new cultivars became closely related after each cycle and resulted in an extremely narrow genetic base of varieties. Any crop with a narrow genetic base is more prone to natural disasters such as the attack of different insects and the outbreak of diseases (Altıntaş et al. 2008). The limited genetic basis of the soybean and peanut cultivars may also be attributed to the contribution of fewer than 20 introducing plant materials, most with unknown pedigree information. The genetic similarity in soybean and peanut genotypes had reached a level that caused a major hindrance for future genetic improvement of soybean and peanut varieties with the aim of enhancing yield and quality.

In light of the narrow germplasm base of soybean and peanut genotypes grown in Turkey, a renewed emphasis should be placed on new sources of germplasm in the breeding pool in order to enhance genetic variability to permit continued progress in developing high yielding cultivars. Future parent selection for crossing should consider using genotypes of greater genetic distance and avoiding genotypes of common background. Plant introduction should be made from countries of greater genetic diversity for these crops. China and neighbouring countries are the center of origin of soybean and similarly South

America for peanut. These areas represent a primary source of germplasm for these crops. Therefore, plant materials should be introduced from China, Korea, Japan, and India for soybean and South America for peanut. Soybean and peanut breeders of other countries have utilized different strategies in their breeding programs. Cui et al. (2000) reported that Chinese soybean breeders avoid mating related parents and continue to introgress new germplasm in cultivar development programs. Satyavathi et al. (2006) reported that the high diversity of Indian soybean cultivars is due to mutation breeding and the use of local landraces collected from the traditional soybean growing region of northern and northeastern part of the country. Recently, Varshney et al. (2009) showed high genetic variations in peanut germplasm including landraces, mutant lines, and cultivars. Wild species and ancestors of cultivated soybean and peanut and exotic germplasm are also important sources of many valuable genes. Fu et al. (2007) found that exotic germplasm had high genetic variation when compared with Canadian cultivars. Therefore, utilization of related wild species or use of exotic material and landraces for development of elite cultivars will be an important approach to create diversity in Turkish soybean and peanut breeding for improving grain yield and quality in the future.

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