International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume:04, Issue:01 "January-February 2018"

ASSESSMENT OF GENETIC DIVERSITY AMONG TURKISH SESAME (*Sesamum indicum L.)* GENOTYPES USING ISSR MARKERS

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ABSTRACT

Information about genetic diversity is very crucial in plant breeding program because of crosses between parents with high inter-parental diversity may also help to develop varieties with broad genetic base. The genetic diversity and the relationships among 24 Turkish sesame genotypes were evaluated using 14 ISSR primers. The 14 ISSR primers amplified 68 bands of which 46 were polymorphic (68.2%). The polymorphism information contents (PIC) value, is the discriminatory power of the primers and used as a relative measure level of polymorphism, ranged from 0.06 0.70 with an average of 0.32. The 24 sesame genotypes were divided into 3 groups in a UPGMA based dendogram. Cluster A included 9 sesame population from different region of Turkey, while cluster B included only 5 sesame population from Adana province and two commercial cultivars (Muganli-57 and Baydar-2001). As to cluster C consist of 5 commercial cultivar which registered by same institute and three sesame population from east part of Turkey. This study gives some useful information about the genetic diversity of 24 sesame landraces. The variation harbored by these landraces could be used in sesame breeding program in Turkey as well as in the world. ISSR primers grouped some landraces based on their geographical provenances and while cluster others showing that ISSR could be useful in geographical clustering of sesame landraces.

Keywords: Sesame, Genetic Diversity, Landraces, ISSR

INTRODUCTION

Sesame (*Sesamum indicum* L. - Pedaliaceae) is one of the oldest and important oil seed crops known to mankind. The genus *Sesamum* contains more than 30 species of which *S. indicum* is the commonly cultivated in the World (Nayar & Mehra 1970; Kobayashi *et al*. 1990; Akbar *et al.* 2011). Sesame seeds are used on bread, cakes and especially used classical diet such as simit in Turkey. The seed is can also be made into a paste called tahini which is rich in protein and a very good energy source and confection called halvah. Sesame was grown during the ancient

ISSN: 2455-6939

Volume:04, Issue:01 "January-February 2018"

Harappan, Mesopotamian, and Anatolian eras for its edible seed and its oil (Bedigian, 2004) but now it is grown in more than 60 countries. There are great number sesame landraces which are adapted to various ecological conditions throughout the Turkey (Demir, 1962).

Despite sesame has been grown during hundred years in Turkey, sesame yield and production is very low. Turkey sesame production is around 16.000 tons from 25.000 ha of and average seed yield is 623 kg ha^{-1}(FAO,2014). One of the most important reasons for low seed yield and production under cultivation is lack of improved varieties in Turkey. This situation can be changed by selecting varieties of good quality and high adaptive potential to the different climatic conditions (Nyongesa *et al* 2012). Sesame landraces are an important source of genetic diversity for breeders. Information about genetic diversity is very crucial in plant breeding program because of crosses between parents with high inter-parental diversity may also help to develop varieties with broad genetic base (Singh 1990; Keneni *et al.* 1997; Keneni et al. 2005). The insufficient genetic information about the Turkish sesame populations is the main factor for developing elite varieties (Baydar *et al*. 1997; Ercan *et al*. 2004). Because landraces have considerable breeding values as they contain valuable adapted genes for differences environment conditions.

Genetic diversity is studied by using various methods such as morphological, biochemical and molecular markers. However, morphological and biochemical markers are affected by environmental factors (Alsaleh *et al.* 2016). Molecular markers overcome this limitation. Molecular markers techniques such as amplified fragment length polymorphism (AFLP) (Ali *et al.* 2007; Frary *et al.* 2015), random amplified polymorphic DNA (RAPD) (Ercan *et al*. 2004; Pham *et al*. 2011; Tabatabaei *et al*. 2011; Patil *et al*. 2016), sequence-related amplified polymorphism (SRAP) (Zhang *et al.* 2010; Zhang *et al.* 2012) , simple sequence repeats (SSR) (Gebremichael & Parzies 2010; Yepuri *et al.* 2013; Nweke *et al.* 2014) , inter simple sequence repeats (ISSR) (Anitha *et al.* 2010; Kumar *et al.* 2012; Alemu *et al*. 2013) and combination of molecular markers (Sharma *et al.* 2009; Kumar & Sharma 2011; Singh *et al.* 2015) have been widely used in genetic diversity studies in sesame.

The aim of the present study was to estimate genetic diversity and relationship in commercially cultivated sesame genotypes using ISSR markers between 24 sesame landraces and cultivars which collected from different region of Turkey and cultivars and to make a strategy for broadening the genetic base for future breeding of these crop.

ISSN: 2455-6939

Volume:04, Issue:01 "January-February 2018"

MATERIALS AND METHODS

Plant Material and Dna Extraction

Twenty-four sesame landraces and cultivars were used in this study from different region of Turkey (Table 1). Genomic DNA was extracted from leaf tissue of 3 three weeks old by the CTAB method of Doyle and Doyle (1990) with minor modifications of Alsaleh *et al*. (2015). The concentration of extracted DNA was estimated by comparing band intensity with lambda DNA of known concentration, after 0.8% agarose gel electrophoresis and ethidium bromide staining. DNA was diluted to 5 ng μL^{-1} for ISSR analysis.

Table 1: The list of 24 sesame landraces and cultivars commonly grown in Turkey

Region	Genotype Name	Region	Genotype Name
South	Kahramanmaras	South	Adana-Saricam
South	Antalya-Kumluca	South	Adana-Yumurtalik 1
West	Aydın-Cine	South	Adana Merkez
South	Adana-Kozan	South	Adana-Yumurtalik 3
Northwest	Balikesir-Koseler	South	Adana-Yumurtalik 7
Southeast	Diyarbakir-Silvan		Baydar-2001 ©
South	Osmaniye		Muganli-57 ©
West	Manisa-Salihli		Kepsut-99 ©
West	Mugla-Ortaca		Osmanli-99 ©
Southeast	Sanliurfa-Bozova-Cukurkoy 2		Orhangazi-99 ©
Southeast	Diyarbakir-Bismil-Bakacak 2		Tan-99 ©
West	Manisa-Alasehir-Ulubentdere		Cumhuriyet-99 ©

©: Cultivar

Issr Analysis

ISSR amplification reaction was applyed according to Baloch *et al.* (2015), using 14 ISSR primers for sesame. The details of ISSR primers are briefly shown in Table 2. Amplification reactions for ISSR were performed in a 25 µL reaction mixture containing 75 mM Tris-HCl, pH 8.8; 20 mM $(NH4)_2SO_4$; 2.0 mM $MgCl_2$; 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.

The PCR reactions were performed in a thermal cycler using the (Techne-412, Barloworld Scientific) following PCR profile: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. ISSR 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 bands were scored manually as present (1) or absent (0) for each primer by two independent researchers. For each primer amplified bands, polymorphic bands and polymorphism percentage (P%) were calculated (Table 2). 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.

RESULTS

The genetic diversity and the relationships among 24 sesame genotypes were evaluated using 14 ISSR primers, Table 2 shows the pattern of amplified and polymorphic bands and polymorphism information contents for each primer. The 14 ISSR primers amplified 68 bands of which 46 were polymorphic (68.2%). The highest number of total bands were amplified as 8 with UBC 827 primer while lowest number of total amplified bands were 3 with three primer UBC 808, UBC 826 and UBC 840 with average of 5.2 bands per primer. The highest number of polymorphisms was observed with primer UBC 826 and UBC 851(100%), while UBC 840 and UBC 848 (33%) harbored the lowest number of polymorphic bands (Table 2).

Table 2: The number of amplified, PIC values and polymorphic bands produced by ISSR primers among 24 sesame landraces and cultivars commonly grown in Turkey.

Primer Name	Sequence (5′-3′)	Annealing Temperature	Amplified Bands	Polymorphic Bands n	P %	PIC Value
UBC 808	$(AG)_8$ C	58	3	2	67	0,06
UBC 811	$(GA)_8$ C	52	5	3	60	0,23
UBC 826	$(AC)_8$ C	52	3	3	100	0,70
UBC 827	(TG)8 A	52	8	6	75	0,21
UBC 834	$(AG)_8$ YT	54	6	4	67	0,43
UBC 836	$(AG)_8$ YA	52	4	3	75	0,21
UBC 840	$(GA)_8$ YT	52	3	1	33	0,30
UBC 848	$(CA)_8$ RG	58	6	2	33	0,16
UBC 851	$(GT)_8$ YG	54	4	4	100	0,74
UBC 854	$(TC)_8$ RG	54	7	4	57	0,08

UBC 855	$(AC)_8$ YT	52	6	3	50	0,28
UBC 868	$(GAA)_6$	48	7	6	86	0,45
UBC 880	GGA GAG GAG AGG AGA	48	6	5	83	0,33
Total			68	46		
Average			5,2	3,4	68,2	0,32

P: Polimorphism; PIC: Polimorphism Information Content

The number of amplified polymorphic fragments ranged from 2 to 6 with an average of 3.4 polymorphic fragments per primer. Polymorphism percentage of the primers was not too low and ranged from 33% to 100% with an average of 68.2%.

According to the Jaccard's similarity index, similarity index values varied between varieties, lines and cultivar ranged from a minimum of 0.02 for varieties Baydar-2001-Muganli-57 to a maximum of 0.46 between Antalya-Kumluca-Diyarbakir-Bismil-Bakacak2.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
2	0,39																						
3	0,41	0,24																					
4	0,24	0,37	0,29																				
5	0,09	0,40	0,38	0,16																			
6	0,15	0,38	0,33	0,16	0,10																		
7	0,16	0,37	0,29	0,15	0,11	0,11																	
8	0,23	0,30	0,24	0,13	0,18	0,18	0,14																
9	0,21	0,38	0,24	0,20	0,20	0,22	0,17	0,14															
10	0,12	0,43	0,38	0,22	0,10	0,10	0,11	0,21	0,17														
11	0,13	0,46	0,36	0,26	0,16	0,17	0,21	0,27	0,13	0,14													
12	0,10	0,36	0,33	0,17	0,11	0,11	0,13	0,16	0,13	0,11	0,12												
13	0,12	0,34	0,32	0,22	0,12	0,09	0,11	0,20	0,18	0,12	0,16	0,08											
14	0,27	0,44	0,25	0,17	0,22	0,22	0,11	0,19	0,13	0,20	0,23	0,20	0,18										
15	0,19	0,38	0,29	0,12	0,14	0,17	0,10	0,11	0,19	0,17	0,24	0,13	0,17	0,11									
16	0,10	0,38	0,33	0,20	0,11	0,08	0,06	0,19	0,17	0,08	0,15	0,09	0,05	0,17	0,16								
17	0,23	0,34	0,27	0,15	0,21	0,19	0,09	0,12	0,20	0,21	0,30	0,18	0,16	0,15	0,11	0,14							
18	0,16	0,37	0,29	0,15	0,11	0,11	0,03	0,14	0,19	0,11	0,21	0,15	0,11	0,11	0,10	0,06	0,09						
19	0,18	0,36	0,28	0,14	0,13	0,13	0,05	0,12	0,17	0,13	0,22	0,14	0,10	0,09	0,08	0,08	0,08	0,02					
20	0,16	0,24	0,30	0,19	0,19	0,19	0,16	0,11	0,23	0,19	0,27	0,14	0,18	0,27	0,17	0,16	0,19	0,16	0,14				
21	0,19	0,34	0,32	0,18	0,20	0,20	0,13	0,17	0,23	0,20	0,26	0,21	0,17	0,18	0,16	0,15	0,13	0,10	0,08	0,19			
22	0,22	0,33	0,30	0,22	0,23	0,21	0,16	0,14	0,22	0,21	0,27	0,24	0,20	0,21	0,17	0,19	0,15	0,13	0,12	0,19	0,07		
23	0,29	0,21	0,23	0,28	0,30	0,28	0,22	0,17	0,24	0,30	0,36	0,30	0,25	0,27	0,24	0,23	0,21	0,18	0,17	0,25	0,15	0,13	
24	0,15	0,35	0,33	0,16	0,15	0,18	0,14	0,15	0,24	0,18	0,22	0,17	0,18	0,19	0,08	0,17	0,16	0,11	0,10	0,17	0,08	0,08	0,18

1 Karamanmaraş;2 Antalya-Kumluca;3 Aydın-Çine; 4 Adana-Kozan;5 Balıkesir-Köseler;6 Diyarbakır-Silvan;7 Osmaniye; 8 Manisa-Salihli;9 Muğla-Ortaca;10 Şanlıurfa-Bozova-Çukurköy2 ;11 Diyarbakır-Bismil-Bakacak2;12 Manisa-Alaşehir-Ulubentdere;13 Adana-Sarıçam;14 Adana-Yumurtalık1;15 Adana-Merkez;16 Adana-Yumurtalık-17 Adana-Yumurtalık7; 18 Baydar-2001; 19 Muganlı-57; 20 Kepsut-99; 21 Osmanlı-99; 22 Orhangazi-99; 23 Tan-99; 24 Cumhuriyet-99

Figure 1: The estimation of Jaccard genetic similarity through combined data ISSR among 24 Turkish sesame landraces and cultivars

ISSN: 2455-6939

Volume:04, Issue:01 "January-February 2018"

From these coefficient values, it was concluded that Baydar-2001 ile Muganlı-57 were close related populations, Antalya-Kumluca ve Diyarbakır-Bismil-Bakacak 2 were genetically distant, UPGMA dendrogram was constructed using genetic similarity index and shown in Figure 2, Cluster analysis differentiated all 17 sesame landraces into three A, B and C, Group A harbored highest number of 9 landraces while group B and C enjoyed 7 and 8 genotypes.

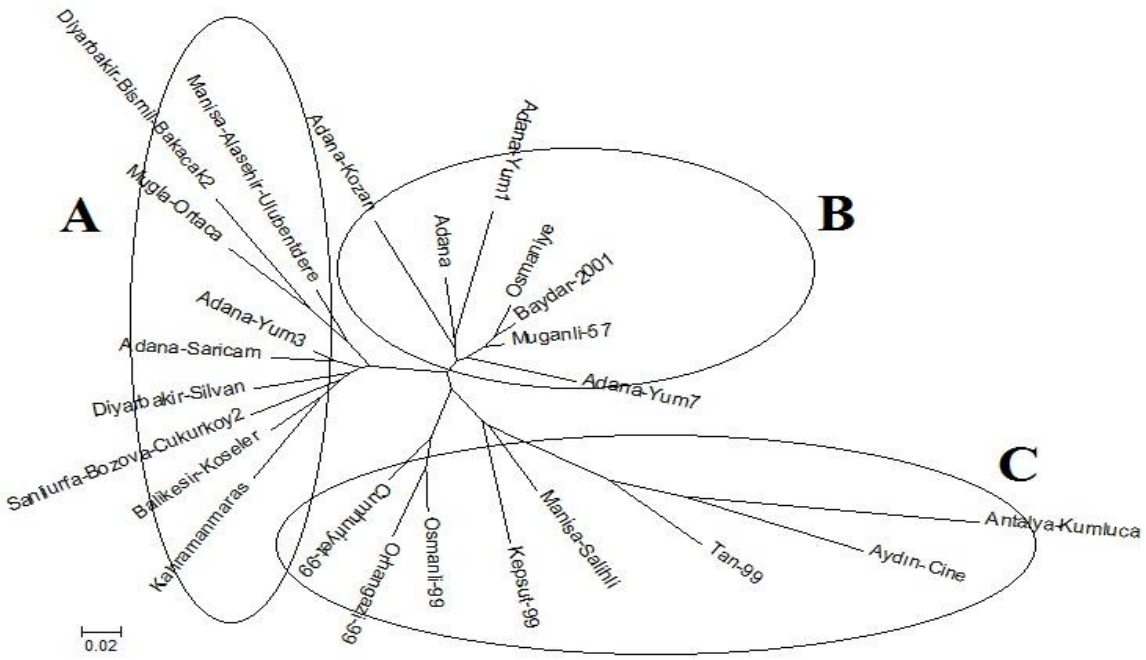

Figure 3: Association among 24 Turkish sesame landraces and cultivars as revealed by UPGMA cluster analysis of Jaccard genetic similarity coefficent calculated from data of ISSR

DISCUSSION

Knowledge of genetic diversity among sesame landraces play a key role in breeding programs to improve grain yield, oil and provide valuable information that can be used by plant breeders. Genetic variability in crop species can be determined using agro-morphological, isozyme and molecular markers. Among the molecular markers, ISSRs markers are very important to study genetic diversity in plant species, as they are effective in detecting very low levels of genetic variation (Baloch *et al.* 2017). The level of polymorphism obtained in this study (68.2%), was high compared with previous studies in which a low level of polymorphism was detected among 75 Korean sesame genotypes (33%) with 14 ISSR markers (Kim *et al.* 2002), 96 world sesame accession (Ali *et al.* 2007) and Indian sesame varieties (57%) (Kumar & Sharma 2011), However, our results were comparable with the 70,6% of polymorphism reported in analysis of

ISSN: 2455-6939

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genetic diversity in Iranian sesame genotypes by Parsaein *et al* (2011) and Dossa *et al.* (2016) detected 70.1% polymorphism among 22 countries sesame genotypes with 33 SSR markers . In Turkey, Ercan *et al*. (2004) used 12 RAPD primers for testing the diversity harbored by 38 Turkish sesame accessions and they found polymorphism level of 78%. The level of polymorphism obtained in this study (%68,2) was low as compared with the 97,3% of Alemu *et al.* (2013) in molecular diversity in six genotypes of north-western Ethiopia and 98,5% of Anita *et al.* (2010) in 10 sesame varieties of Tamil Nadu. The difference in polymorphism could be due to the genotypes used, nature of primers used and annealing temperatures of the primers. Low annealing temperature may increase non-specific amplification, leading to artefact bands. The modification of annealing temperature has a great impact on the richness and legibility of fingerprints (Bornet *et al*. 2001).

The polymorphism information contents (PIC) value, is the discriminatory power of the primers and used as a relative measure level of polymorphism, ranged from 0.06 for primer UBC 808 to 0.70 for primer UBC 826 with an average of 0.32. Four primers produced polymorphism information contents value greater than 0.4 which showed that ISSR markers were not enough powerful for discriminating the population. These PIC values higher than by Laurentin *et al.* (2007) and Zhang *et al.* (2012) and lower than reports of Dixit *et al*. (2005), Cho *et al.* (2012), Yepuri *et al.* (2013) and Dossa *et al.* (2016). The differences observed with other studies might be due to the use of different accessions and the number and type of molecular markers.

The 24 sesame genotypes were divided into 3 groups in a UPGMA based dendogram (Fig. 3). Cluster A included 9 sesame population from different region of Turkey, while cluster B included only 5 sesame population from Adana province and two commercial cultivars (Muganli-57 and Baydar-2001). As to cluster C consist of 5 commercial cultivar which registered by same institute (Aegean Agricultural Research Institute) and three sesame population from east part of Turkey. Grouping of the sesame genotypes were partially according to geographical provinces, which show that ISSR markers have good discrimination power for grouping sesame accessions according to their collection site. Results of the Neighbor-Joining tree showed three major cluster groups. Studied genotypes are randomly distributed in group A and it seems that these materials have been exchanged among farmers of different regions. While genotypes are not randomly distributed in group B and C (Fig. 3). In agreement these results, Ali *et al.* (2007), also found relationship between genetic diversity and geographical distribution in 96 sesame accessions from different part of world. Although, some earlier reports are not found geographical distribution in different sesame accession (Abate *et al* 2015; Singh *et al.* 2015; Zhang *et al.* 2010; Sharma *et al.* 2009; Kumar & Sharma, 2011).

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CONCLUSION

This study was given some useful information about the genetic diversity of 24 sesame landraces. The variation harbored by these landraces could be used in sesame breeding program in Turkey as well as in the world. ISSR primers grouped some landraces based on their geographical provenances and while cluster others showing that ISSR could be useful in geographical clustering of sesame landraces. However, this study used a small sample size, geographic range, and limited primers. Therefore, to find clear patterns of diversity for the whole country and to reach a sound conclusion, further studies should be conducted with large sample sizes and geographic range using more ISSR primers and using SSR markers as well as GBS based markers.

ACKNOWLEDGEMENT

This study was supported by the the Scientific Research Projects Coordination Unit of Cukurova University (ZF2009D40).

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ISSN: 2455-6939

Volume:04, Issue:01 "January-February 2018"

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