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GENETIC DIVERSITY OF OKRA (*ABELMOSCHUS ESCULENTUS* L.) GENOTYPES FROM DIFFERENT AGRO-ECOLOGICAL REGIONS REVEALED BY AMPLIFIED FRAGMENT LENGTH POLYMORPHISM ANALYSIS

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ABSTRACT

This study was carried out to assess the genetic diversity in 48 genotypes and accessions using eight Amplified Fragment Length Polymorphism (AFLP) primer-pairs. The eight selected AFLP primer-pairs generated a total of 150 polymorphic loci. Using the generated AFLP data, the Un-Weighted Pair Group Method with Arithmetic Average (UPGMA) ordered the genotypes into six groups based on Dice similarity coefficient. The range in taxonomic distance was from 0.23 to 1.0. Each cluster was found to have genotypes and accessions from different regions and climate and sometimes different continents. The size range of the loci ranged from 87-662 bp. Great variation between the genotypes and accessions in the different cluster could be of high value as the genetically diverse okra genotypes represent a potentially valuable source for improved pathogen and pest resistance.

Keywords: Amplified Fragment Length Polymorphism (AFLP), UPGMA, Okra Genotypes, Genetic Variation

1. INTRODUCTION

Okra is a member of the *malvaceae* family, which includes fiber crops such as cotton (Gos*sypium spp*) and kenaf (*Hibiscus cannabianus*). The present accepted binomial is *Abelmoschus esculentus* (L.) Moench (Siemonsa, 1982), formerly *Hibiscus esculentus* L. (Borssum Waalkes, 1966; Bates, 1968). The genus *Abelmoschus* comprises nine species (IBPGR, 1990). It is a traditional vegetable crop in many tropical, subtropical and Mediterranean countries. The origin of okra remains unclear but centers of genetic diversity include West Africa, India and Southeast Asia (Charrier, 1984; Hamon and Van Sloten, 1989).

Van Borssum-Waalkes distinguished only six species: three cultivated (*A. moschantus*, *A. manihot* and *A. esculentus*) and three wild (*A. ficuleus*, *A. crinitus* and *A. angulosus*). Kundu and Biswas (1973; Terell and Winters, 1974) distinguished the genus *Abelmoschus* from *Hibiscus*. Okra is increasing in popularity and is now commonly available as a boiled or fried vegetable dish at restaurants salad bars and cafeterias. Fresh tender fruit provide dietary fiber, protein and vitamin C in human nutrition (Candlish *et al*., 1987). Okra seeds have also gained much interest as a new oil and protein source (Düzyaman, 1997).

However very little information is available about cytogenetics and reproductive biology of this very important vegetable crop. Phenotypic variation has been studied by several studies (Salameh and Kasrawi, 2011; 2007; Bello *et al*., 2006; Düzyaman, 2006; Ghai *et al*., 2005; Rawashdeh, 1999; Ariyo, 1987). Nuclear DNA content of okra using Flow Cytometry has been measured to be ranged between 3897-17321 Mpb (Salameh, 2014).

Without a broad base of heterogeneous plant material, it is impossible for plant breeders to produce cultivars that meet the changing needs regarding adaptation to growing conditions, resistance to biotic and a biotic stresses product yield or specific quality requirements (Friedt *et al*., 2007). Therefore, the most efficient way to farther improve the performance of crop varieties is to access to large diverse pool of genetic diversity.

In recent years molecular markers and especially DNA-based markers, have been extensively used in

many areas such as gene mapping and tagging (Kliebenstein *et al*., 2001) characterization of sex, (Flachowsky *et al*., 2001), analysis of genetic diversity (Erschadi *et al*., 2000), or genetic relatedness (Mace *et al*., 1999). DNA based methodologies are now the method of choice to differentiate closely related organisms (Widen *et al*., 1994). Rawashdeh (1999) reported significant differences between 19 local landraces of Jordan using RAPD. To overcome the limitation of reproducibility associated with RAPD, AFLP technology (Vos *et al*., 1995) was developed. A method that has become widely applied in plant population genetics.

Knowledge of genetic diversity of a species has an important impact on the improvement of crop productivity as well as the conservation of genetic resources. In recent years more attention has been given to the genetic analysis of diverse genotype sets, which are particularly attractive for association analysis of qualitative traits such as disease resistance or special quality characteristics (Hasan *et al*., 2006). To our knowledge, scarcely studies have been focused on studying the genetic diversity at DNA level of okra benefiting from the advantages of AFLP. Therefore this research aims to estimate the genetic relationship between 48 okra genotypes from different agro-ecological regions using the AFLP marker.

2. MATERIALS AND METHODS

A total of 48 seed samples of okra genotypes and accessions were obtained from National Center for Agricultural Research and Extension (NCARE) Amman-Jordan and The World Vegetables Center/Taiwan, together with a commercial check cultivar (Fairooz) (**Table 1**). Okra seeds were grown in the greenhouse (Plant Breeding Department, Giessen, Germany). In order to take into account possible genetic variability within each accession, total genomic DNA was extracted from bulked young leaves (100-200 mg per accession) of ten 4-5-week-old plants following the CTAB procedure according to Doyle and Doyle (1990). After RNAse treatment, DNA content was fluorometrically quantified (DynaQuant 200 Hoefer Scientific Instruments) and diluted to 25 ng μ^{-1} working solution. AFLP analysis was performed according to Vos *et al*. (1995) by using the Invitrogen AFLP® Core Reagent Kit following the manufacturer's instructions. Here, 125 ng of genomic DNA (i.e., 5 μL of working solution) were digested using *EcoR*I and *MseI* restriction enzymes and generated fragments were ligated with double-stranded site-specific adapters using T4 DNA ligase. Ligation was followed by two pre-amplifications (+0, +1) prior to the final amplification phase performed by using primer combinations having three selective nucleotides.

Table 1. List of 48 okra (*Abelmoschus esculentus* L.) genotypes and accessions with their region together with the control cultivar

S/number	Landrace/origin	Source
1	Sade/India	Dr. Düzyaman[1]
2	Sultani/Turkey	Dr. Düzyaman
3	Bati Trakya I/Turkey	Dr. Düzyaman
4	Bati Trakya II/Turkey	Dr. Düzyaman
5	Balikesir T1/Turkey	Dr. Düzyaman
6	Prabhani Kranm/India	Dr. Düzyaman
7	Genin/Palestine	Dr. Düzyaman
8	Denizli/Turkey	Dr. Düzyaman
9	Amasya/Turkey	Dr. Düzyaman
10	Sultani May/Turkey	Dr. Düzyaman
11	UGA red/USA	Dr. Düzyaman
12	Aglasin Burdur/Turkey	Dr. Düzyaman
13	Lee/USA	Dr. Düzyaman
14	Kabakli/Turkey	Dr. Düzyaman
15	Akkoy/Turkey	Dr. Düzyaman
16	JOR 84/Jordan	NCARE[2]
17	JOR 169/Jordan	NCARE
18	Egy 1/Egypt	Private communication
19	Egy 2/Egypt	Private communication
20	Egy 3/Egypt	Private communication
21	JOR 48/Jordan	NCARE
22	JOR 3/Jordan	NCARE
23	JOR 1/Jordan	NCARE
24	JOR 42/Jordan	NCARE
25	TOT 3886/Thailand	AVRDC[3]
26	JOR 8/Jordan	NCARE
27	JOR 52/Jordan	NCARE
28	JOR 12/Jordan	NCARE
29	TOT 7101/Philipines	AVRDC
30	Commercial cv (Fairooz)	NCARE
31	JOR 2/Jordan	NCARE
32	JOR 34/Jordan	NCARE
33	JOR 49/Jordan	NCARE
34	TOT 7102/Philipines	AVRDC
35	TOT 7957/USA	AVRDC
36	TOT 7958/USA	AVRDC
37	TOT 7960/USA	AVRDC
38	TOT 7961/USA	AVRDC
39	TOT 7963/Guatemala	AVRDC
40	TOT 7966/Yugoslavia	AVRDC
41	TOT 6214/Thailand	AVRDC
42	TOT 7159/Malysia	AVRDC
43	TOT 2739/Malysia	AVRDC
44	TOT 7164/Mynamar	AVRDC
45	TOT 7219/Malysia	AVRDC
46	TOT 7343/Vietnam	AVRDC
47	TOT 7345/Vietnam	AVRDC
48	TOT 7346/Vietnam	AVRDC

[1]Department of Horticulture, Faculty of Agriculture, Ege University, Turkey
[2]National Center for Agriculture Research and Extension
[3]The World Vegetable Center

The selective amplification mixture (total volume of 25 µL) consisted of 7.5-12.5 ng fluorescent dye-labelled *EcoR*I primer, 30 ng *Mse*I primer, 0.2 mM of each dNTPs, 2 µL PCR buffer, 0.5 U Taq-polymerase (Qiagen, Germany) and 5 µL of pre-amplified PCR-product in deionised distilled water.

Selective ampliWcation products were separated on 8% denaturing polyacrylamide gels using a Li-Cor 4200 DNA Analyzer. Fragments size was estimated incomparison to a 50-750 bp labelled DNA-ladder Scoring and analyses of AFLP data AFLP fragments were detected using the RFLPScan 2.1 software package (Scananalytics, Fairfax, USA). Clear and unambiguous fragments were scored as present (1) or absence (0) to generate a binary data matrix. The number of polymorphic fragments was determined for each primer pair used. Only polymorphic fragments were used for further data analysis. Pairwise relatedness based on genetic similarity (Dice, 1945) was estimated between all okra accessions using the SIMQUAL module of NTSYS pc software version 2.20e (Rohlf, 1993). UPGMA (unweighted pair-grouped method using arithmetic averages) cluster analysis was performed following GenDist and NEIGHBOR programs available in the software package PHYLIP 3.6 (Felsenstein, 1985).

3. RESULTS

3.1. AFLP Data Analysis

In our study, eight AFLP marker combinations were used to profile the 48 okra (*Abelmoschus esculentus* L.) genotypes including the control cultivar Fairooz (**Fig. 1**). As shown in **Table 2** a total of 150 polymorphic loci were generated, ranging in size from 6 to33 bp. The number of amplified loci per primer varied from 6 loci (*Mse*I_GAC and *EcoRI*_ATC) to 33 loci (*Mse*I _CTC and *EcoRI*_ACA

3.2. Cluster and Principal Coordinate Analyses

Dice similarity coefficient (Dice, 1945) which is a matching coefficient for binary data generated AFLP analysis, was used to cluster the 48 okra (*Abelomschus esculentus* L.) genotypes together with the control cultivar Fairooz with the Unweighted Pair Group Method with Arithmetic Average (UPGMA). The 48 genotypes felt into 6 groups (**Fig. 2**). The similarity level between genotypes and accessions in cluster I ranged from 0.56 to 0.83. The highest genetic similarity percentage (0.83) was observed between (Sade) and (TOT 7963/Guatemala), while the lowest level (0.56) was found to be between (Bati Trakya I/Turkey) and the rest of the genotypes and accessions in the same cluster.

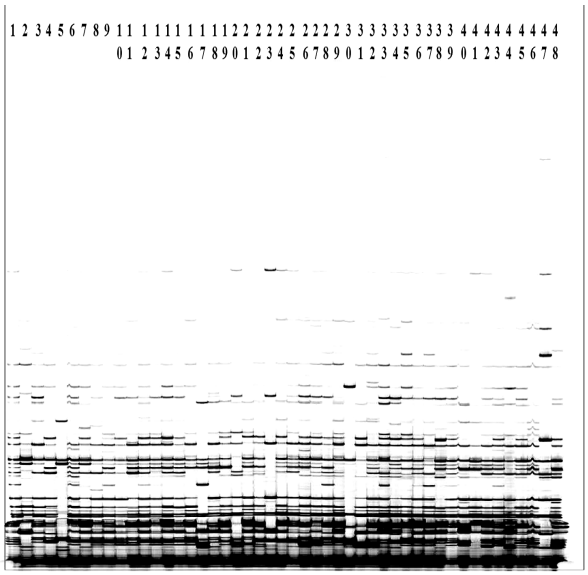

Fig.1. AFLP profiles of the 48 okra genotypes and accessions by the primer combination *MseI*-CTC/*EcoRI*-AGC. Lanes 1 to 48 presents: (1: Sade; 2: Sultani; 3: Bati Trakya I; 4: Bati Trakya II; 5: Balikesir T1; 6: Prabhani Kranm; 7: Genin; 8: Denizili; 9: Amasya; 10:Sultani May; 11: UGA red; 12: Aglasin Burdur; 13: Lee, 14: Kabakli; 15: Akkoy; 16: JOR 84; 17: JOR169; 18: EGY1; 19: EGY2; 20: EGY3; 21: JOR 48; 22: JOR 3; 23: JOR1; 24: JOR 42; 25: TOT3886; 26: JOR 8; 27: JOR 52; 28: JOR 12; 29: TOT 7101; 30: FAIROOZ CV; 31: JOR 2; 32: JOR 34; 33: JOR 49; 34: TOT 7102; 35: TOT 7957; 36: TOT 7958; 37: TOT 7960; 38: TOT 7961; 39: TOT 7963; 40: TOT 7966; 41: TOT 6214; 42: TOT 7159; 43: TOT 2739; 44: TOT 7164; 45: TOT 7219; 46: TOT 7343; 47: TOT 7345; 48: TOT 7346

While in cluster II, the similarity level between the genotypes and accessions ranged from (0.44) to (0.62). The higher genetic similarity percentage was found between (Sultani May/Turkey) and (Kabakli/Turkey) while the lowest similarity coefficient (0.44) was between (JO 12) and (JO 49). Cluster III, which consists of only three genotypes, the highest similarity coefficient (0.45) was observed between (Egypt I) and (TOT 7102/Philippines). Cluster IV, the similarity coefficient ranged between 0.4 to 0.42, the highest was found between (UGA red) and (JO 84), while the lowest similarity coefficient was found between (TOT7346/Vietnam) and the rest of the cluster IV. Cluster V indicated the highest similarity coefficient (0.44) to be between (Egypt II) and (TOT 6214/Thailand), while the lowest coefficient was found to be (0.2) between (JO 169) and the rest of the clusters genotypes and acessions.

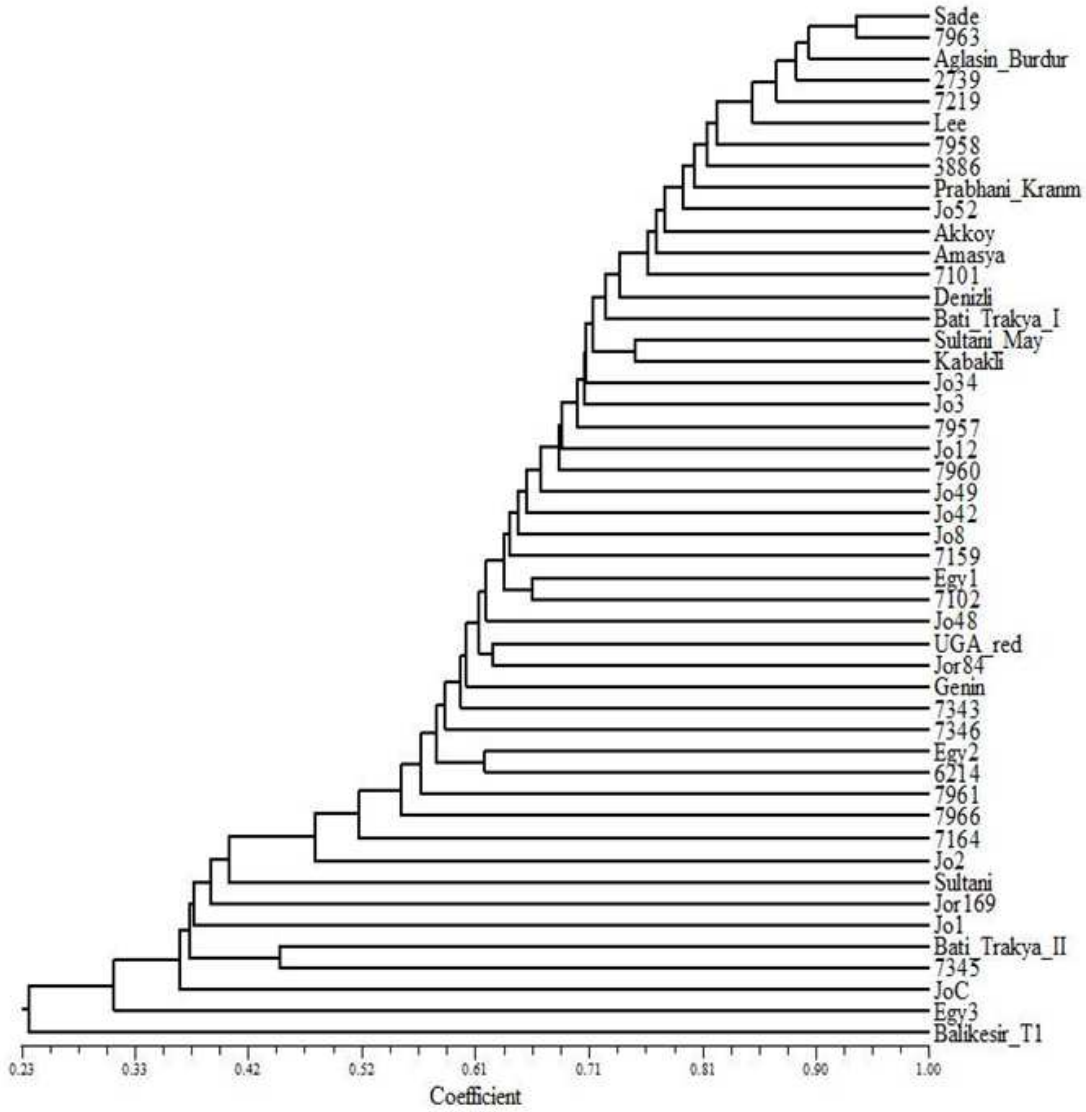

Fig. 2. Dendogram generated based on UPGMA clustering method and Dice coefficient using AFLP analysis among 48 okra genotypes and clusters

Table 2. Standard statistics for the AFLP primer combination tested on the 48 okra (*Abelmoschus esculentus* L.) genotypes and accessions togethe with the control cultivar (Fairooz)

Primer combination		Number of polymorphic loci	Size range
*MseI*_ AGC	*EcoRI*_CCG	21	87-359
*MseI*_CCA	*EcoRI*_AGA	31	137-662
*MseI*_CCA	*EcoRI*_AGC	8	201-334
*MseI*_CCA	*EcoRI*_ATG	19	121-416
*MseI*_CTC	*EcoRI*_ACA	33	112- 609
*MseI*_CTC	*EcoRI*_AGC	15	100-261
*MseI*_GAC	*EcoRI*_ATC	6	153-341
*MseI*_GTG	*EcoRI*_AAA	17	161-434
Total/range		150	87-662

The last cluster (Cluster VI) has been found to consist of only six landraces including the commercial cultivar Fairooz, The highest similarity (0.30) between (Bati Trakya II/Turkey) and (TOT 7345/Vietnam), while the lowest (0.14) was found between (Balikesir T1/Turkey) and the rest five landraces in the same cluster.

4. DISCUSSION

Forty eight genotypes and accessions of okra has been collected from different regions with the aim to measure genetic distinctiveness using AFLP markers. The result of the study demonstrated the suitability of AFLP data for the analysis of genetic diversity in *Abelmoschus esculentus* L. genotypes and accessions. Unlike genetic diversity, diversity based on phenotypic and morphological characters, usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification, but now the rapid development of biotechnology allows easy analysis of large number of loci distributed throughout the genome of the plants (Chakravarthi and Naravaneni, 2006). Information on the genetic diversity in okra (*Abemoschus esculentus* L.) collections can give breeders and geneticists important information on the allelic diversity present in genebank materials and may help to identify genetically diverse pools for use in cross combinations to improve important agronomic traits or to better exploit heterosis (Diers and Osborn, 1994).

AFLP is a powerful fingerprinting technique, which detects polymorphism on the level of restriction enzymes sites. It is based on PCR amplification of restriction enzymes and oligonucleotides adaptors of few nucleotide bases. This method generates a large number of restriction fragment bands facilitating the detection of polymorphism. Therefore, AFLP markers combine the advantages of RFLP's and PCR-based markers. This technology has been adopted for fingerprinting and mapping of different plants (Hussein *et al*., 2005) as well as some pathogens (Meza-Moller *et al*., 2011). Omahinmin and Osawaru (2005) reported that high degree of wide morphological variation exist among accession of okra which requires further evidence using molecular markers to clarify. Molecular markers have been successfully used in the genus *Abelmoschus* to select parents for hybrid production, for intra-specific or inter-specific classification and for the analysis of variation. Akash *et al*. (2013) indicated a lack of significant correlation between phenotypic and actual AFLP genetic profile inferred by UPGMA clustering in 21 landraces of Jordanian okra (*Abelmoschus esculentus* L).

In conservation programs for plant genetic resources, the availability of characterization data and information on available genetic diversity can help germplasm users to identify accessions of interest and also provide plant breeders with initial data regarding materials for use in crop improvement programs (Cruz *et al*., 2007). The Asian accessions was found to be more diverse than the accessions from African or USA, this could be mainly due to that the accession have be collected from different geographical regions from East to South of the continent, this result agress with Aladele *et al*. (2008) who indicated more diversity among the Asian genotypes using RAPD markers, this variation could be due to that the genotypes were originally collected from six different countries in the region. From this study, the Jordanian accessions were found to be diverse as they have been located in different clusters, the results agree with Kaur *et al*., (2013) who found great diversity between okra accession using RAPD. Many studies using Cluster analysis showed that similarity between the okra lines was from 100 to 15.41%. Gulsen *et al*. (2007) found the relatedness value based on SRAP markers ranging from 100 to 86% among 23 okra genotypes. Aladele *et al*. (2008) observed relatively more molecular diversity in the Asian genotypes as compared to those of African origin. Genetic distance values ranging from 0.00 to 0.66 were observed among okra accessions (Saifullah *et al*., 2010). The present investigation has provided a useful insight into the extent of genetic diversity in the okra germplasm and can be exploited in future breeding programs as well as for the development of mapping populations/linkage map.

The result of AFLP indicate a genetic diversity between the different accessions from different regions (cluster I and VI), this could be of high interest for breeders to start a successful breeding programs.

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