Diversity and relationships among Turkish okra germplasm by SRAP and phenotypic marker polymorphism

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Abstract: Germplasm characterization is essential and molecular markers provide valuable information for breeding programs. Sequence-related amplified polymorphism (SRAP) and phenotypic markers were studied to determine diversity and relationships among 23 okra (Abelmoschus esculentus (L) Moench) genotypes. The 39 combinations of forward and reverse SRAP primers were used to evaluate the 21 Turkish and two randomly selected USA genotypes as outgroups, and produced 97 scorable markers, of which 50% was polymorphic for all 23 genotypes. Seventeen out of the 23 genotypes (74%) were distinguished from each other with mean similarity of 0.93. As to phenotypic markers, 33 heritable traits were evaluated in field with ten replications, 28 of them (85%) were found to be polymorphic. The UPGMA (unweighted-pair group method arithmetic average) dendrogram based on the 33 phenotypic markers distinguished all genotypes, but failed to detect any geographic association of okra genotypes, being consistent with previous study. It can be concluded that SRAP markers are useful for studying diversity and relationships among okra germplasm, and have potential in marker-aided selection, linkage mapping, and evolutionary studies.

Key words: okra; Abelmoschus esculentus; diversity; germplasm; SRAP; phenotypic marker.

Introduction

Okra (Abelmoschus esculentus (L.) Moench), previously named as Hibiscus esculentus (L.) belongs to Malvaceae family. The centers of genetic origin of Abelmoschus spp. are West Africa, India, and Southeast Asia. A. caillei is another okra species which is also consumed as vegetables in Africa. A. esculentus is annual in tropical regions, while A. caillei is grown perennial particularly in West Africa. There are two other wild species of okra, A. moskhatus and A. manihot. Okra is cultivated in tropical, sub-tropical, and temperate zones and it is an important vegetable crop in India, West Africa, Southeast Asia, USA, Brazil, Australia, and Turkey. The world okra production was 4.8 million tons, but the most of the production was in India (70%) followed by Nigeria (15%), Pakistan (2%), Ghana (2%), Egypt (1.7%), and Iraq (1.7%).

Information on the level of diversity for important agronomic traits of okra is limited. Knowledge on genetic diversity and relationships among the okra germplasm may play significant role in breeding programs to improve fruit quality and resistance to biotic and abiotic stresses of okra. Inter-specific hybridization is possible between Abelmoschus spp. (Fatokun 1987; Hamon & Hamon 1991; Akhond et al. 2000). This may accelerate diversity and increase gene pool for breeding programs. Diversity within germplasm is critical for okra breeding program. Ashraf et al. (2002) found differential responses for gas exchange rates, transpiration rate, stomatal conductance, chlorophyll content, and leaf water potential in two okra cultivars subjected to two different water regimes. Martin & Rhodes (1983) indicated that A. esculentus differed from A. caillei for 9 out of 10 seed characteristics, such as weight, color, and oil content. Within species variation among 30 African genotypes was found to be considerably large based on phenotypic markers (Ariyo 1993). Root knot nematode resistance was also highly variable (Thies et al. 1998). Okra flowers are hermaphrodite and heavily self-pollinated. Cross-pollination also occurs depending on frequency of pollen transfer by insects (Hamon & Koechlin 1991). This outcrossing of okra ensures diversity that provides valuable opportunity for further okra improvement and help extended adaptability for local okra genotypes.

Molecular markers for classification of genotypes are abundant, and, unlike morphological markers, are not affected by environment (Staub & Serquen 1996). Molecular markers can be used to identify unique genotypes and associated agronomic traits. Studies using molecular markers in okra are lagging behind the other major species. The only study reported is that of Martinello et al. (2001) by randomly amplified polymorphic
DNA (RAPD) marker. Considerable RAPD marker diversity was found among 39 *Abelmoschus* spp. Although diversity was reported among selected *Abelmoschus* spp., additional tools can make valuable contribution for breeding programs.

Sequence-related amplified polymorphism (SRAP) markers were recognized as a new and useful molecular marker system for mapping and gene tagging in *Brassica* (Li & Quiros 2001). SRAP markers are PCR-based markers that amplify open reading frames and produce a number of co-dominant markers per amplification. SRAPs use forward and reverse primers, 17 or 18 nucleotides long, and primers consist of a core sequence of 13 or 14 bases, at the 5’CCGG in the forward primer and AATT in the reverse primer, targeting open reading frames in genomic sequences. This core sequence is followed by three selective nucleotides at the 3’ end of each primer. SRAP markers are more consistent and repeatable than RAPDs, and are less labor-intensive and time-consuming to produce than amplified-fragment length polymorphism techniques (Welsh & McClelland 1990; Li & Quiros 2001; Ferrio et al. 2003; Budak et al. 2004a,b,c; Gulsen et al. 2005).

Turkey, closely connected to the two major diversity centers of okra, India and Africa, may have considerable level of diversity, as in many other important plant species. Understanding the genetic structure and germplasm diversity present in okra, germplasm may bring valuable information for okra breeding programs. The objectives of this study were to evaluate SRAP and phenotypic markers to determine diversity and relationships among okra genotypes.

**Material and methods**

**Plant materials**

Twenty-one okra genotypes of *Abelmoschus esculentus* (L.) Moench and two USA genotypes as outgroups, USA1 and USA2, were evaluated in this study (Table 1). Twenty-one okras included those collected from different geographic origins in Turkey. All genotypes were selected from a large collection maintained at the Aegean Agricultural Research Institute, Menemen, Izmir, Turkey. For SRAP marker study, a single seed was germinated, and its leaf and meristematic part was used, while ten seeds from each genotype were used for phenotypic marker study.

**Phenotypic characterization**

Evaluations in this study were according to UPOV, FAO 1999 and International Plant Genetic Resources Institute (IPGRI), following the descriptors developed for okra, and the data were converted to numerical values for statistical analysis. The seeds were planted on the 18th of April, 2002. Intra- and inter-row distance was 20 cm × 75 cm. Experimental design was randomized complete design with 10 replications. The study was carried out at Alata Horticultural Research Institute, Mersin, Turkey, located at 36°37’31” north latitude, 34°20’50” east longitude, and 5 m elevation.

The 21 okra genotypes were evaluated for 33 morphological traits that include four main components: (1) phenological evaluations including, all starting from sowing date, germination time (days) GRTM, first true leaf appearance time FTLT (days), first flowering time FFWT (days), and time of first fruit set FFRT (days); (2) plant characters including growth habit GRHT (prostrate, intermediate, erect), branching habit BRHT (weak, medium, strong), stem pubescence STPB (sparse, intermediate, dense), stem color STCL (green, green-red, red), leaf shape LFSH (entire, medium lobed, deep lobed), leaf size LFSZ (small, medium, large), fruit position FRPS (erect, intermediate, pendant), fruit color FRCL (yellowish green, green, green with red stripe, red), fruit diameter (mm) in harvest FRDT, fruit length (mm) in harvest FRLT, fruit shape FRSP (1 to 15 according to descriptor), petiole length in harvest PTCL (mm), surface between ridges on fruit SBRF (concave, flat, convex), fruit surface angularity FRSA (sparce, medium, high, very high), fruit pubescence FRPB (sparse, intermediate, dense), fruit weight FWG (g); and (4) seed characters including seed color SDCL (green, light green, dark green, black), seed shape SDSP (round, heart, kidney, flat rectangular, flat round) and weight of 1000-grain weight SDWG (g).

**DNA extraction**

Total DNA was extracted from 40–50 mg young frozen leaf tissue of individual genotypes, using a DNA extraction kit, Puregene® (Gentra Systems, Minnesota, USA). DNA concentration was measured with a fluorometer (Hoefer Scientific Ins., San Francisco, USA) and 5 ng/µL DNA templates were made using TE (10 mM Tris-Cl, 0.1 mM EDTA, pH 8.0).
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1.00 0.96 0.93 0.89 0.86
Coefficient

Fig. 1. UPGMA dendrogram based on similarity matrix constructed from the 97 SRAP markers amplified for the 21 Turkish and the two USA okra genotypes as outgroups.

PCR parameters and gel analysis
The 39 combinations of 13 forward and 16 reverse SRAP primers previously evaluated in buffalograsses by Gulsen et al. (2005) were used in this study. Each 25 µL reaction consisted of 5 pM/µL of each of primer pairs, 200 µM of each of dNTPs, 2.5 µL of 10 x PCR BufferR, 5 µL of Q SolutionR, 2 mM of MgCl2 as a final concentration, 6 µL ddH2O, and 1 unit of Taq polymerase (Qiagen, Valencia, CA, USA), 25 ng of template. Perkin Elmer Cetus DNA Thermal CyclerR (Shelton, CT, USA) was used and cycling parameters included: one cycle of 2 min at 94°C, 34 cycles of 1 min at 94°C, 1 min at 47°C, 1 min at 72°C, and for extension, one cycle 5 min at 72°C. PCR products were separated on 2.5% agarose gel at 90 volt for 5 or 6 h.

Scoring gels and data analyses
Each band was scored as present (1) or absent (0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package (Rohlf 1993). A similarity matrix was constructed based on Dice’s coefficient (Dice 1945), which considers only one to one matches between two taxa for similarity. The similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average) to determine genetic relationships among the germplasm studied. Unlike molecular markers that show binary distribution, phenotypic marker data have continuous distribution for most traits. Therefore, row data were first standardized using the standardization option nested in NTSYS-pc software package. SIMINT module was used to compute a distance matrix. Then, distance matrix was used to construct a dendrogram based on the UPGMA method. In order to see how well a cluster analysis represents the distance matrix, CEPH module was used to transform the tree matrix to a matrix of ultrametric distances (a matrix of distances implied by the cluster analysis). Finally, MXCOMP module was used to compare these ultrametric distances and distance matrix produced for UPGMA analysis.

Results and discussion

Evaluation of SRAP markers and cluster analysis
A total of 39 combinations of forward and reverse SRAP primers were screened and a total of 97 bands with high intensity were scored. The number of bands scored per primer ranged from 2 to 6. The scored markers were comprised of fragment sizes ranging from 110 to 1400 base pairs. The number of polymorphic bands observed in all 23 genotypes including the two USA genotypes was 49 out of 97 (50%), and 44 out of 97 (45%) in 21 Turkish okra genotypes. The number of markers scored per genotype for the 39 combinations of primers ranged from 65 to 78 with the average of 71. Cophenetic correlation between ultrametric similarities of tree and similarity matrix was found to be high (r = 0.94, P < 0.01), suggesting that the cluster analysis (Fig. 1) strongly represents the similarity matrix.

There were two groups that comprised identical genotypes (Fig. 1). The first identical group had genotypes 83, 89, 90, and 100. The second group contained two identical genotypes, 97 and 98. High similarity among the genotypes studied was expected because okra has heavily self pollinated species (Hamon & Koechlin 1991). The remaining 17 genotypes including two outgroups were distinguished from one another. Although about more than a four-fold band number (97) of the genotype number (23) was used in this study, only 17 genotypes were distinguished. This is probably due to heavily self-pollinating nature of okra. SRAP markers were also used to distinguish buffalograss genotypes (Budak et al. 2004b,c) and other cross pollinating species (Phan 2000; Budak et al. 2004a).

The number of polymorphic markers analyzed is important to detect true associations among taxa. To
date, there are no firm guidelines as to the number of markers necessary for a particular use. One general guideline was to use a set that covers the genome (Dudley 1994). Numbers in the 20s were sufficient for developing distance measures. Dudley suggested that when numbers reach 50 to 100 markers, results were consistent with pedigree information. Ninety-seven SRAP bandswere used in this study, and this is within the suggested range by Dudley (1994).

Similarity values among the 23 genotypes including two outgroups ranged from 0.86 to 1.00 (Fig. 1). SRAP markers successfully classified okra genotypes into the meaningful clusters. The outgroups from the USA, USA1 and USA2, grouped separately from the 21 okra genotypes collected from throughout Anatolia. The second meaningful cluster is that of genotypes 104 and 105, which were both collected from the same geographic location. The two USA genotypes, used as outgroups, were grouped within the Turkish genotypes. This may reveal high genetic relationship among the genotypes studied.

Cluster analysis based on phenotypic markers
Thirty-three phenotypic markers were scored for the 21 Turkish okra genotypes and 28 (85%) of them were found to be polymorphic. The data indicated potential for okra improvement for most traits. Out of 28 polymorphic markers, 16 showed continuous variation, having at least 3 or more alternative form of each trait. In a previous study, the 114 genotypes were evaluated for the same 33 traits (not published). This study indicated 31 out of 33 traits had continuous distribution among the 114 Turkish okra genotypes. The ratio of polymorphic phenotypic markers (85%) is higher than that of polymorphic molecular markers (50%) among the same genotypes. This might be due to quantitative nature of most phenotypic markers, such as plant height, seed weight, and flower size, in which more genes are involved compared to qualitative nature of molecular markers (Allard 1999).

Dendrogram based on phenotypic marker data successfully distinguished all 21 okra genotypes from one another (Fig. 2), being consistent with the study of the 114 okra genotypes using the same phenotypic markers (not published). Dissimilarity values among the 21 okra genotypes ranged from 0.07 to 0.39. However, the clusters did not associated with geographic origins of okra genotypes. This is consistent with the previous study of 30 okra genotypes by Ariyo et al. (1987). Molecular and phenotypic marker-based analyses produced two different clustering patterns in this study. This may be caused by quantitative control of phenotypic traits studied, and/or fluctuations in enironmental conditions, having potential effect on phenotypic performances.

Few studies of molecular markers have been reportedin okra. The results of this study may therefore give valuable insights to okra genome. They indicate that SRAP markers may be used in improving cultivars, understanding relationships, establishing germplasm collections, integrating markers into genetic linkage maps, and establishing germplasm core collections. For example, okra is harvested every 3 to 5 days during harvest season, and pesticide-free okra is crucial to consumer health. Therefore, resistant or tolerant cultivars to pests are critical. Aphids, for example, cause yield loss and low quality fruits due to altered fruit and leaf structure in plants (Panda & Khush 1995). SRAPs and some phenotypic markers, such as pubescence, may have potential in developing resistant cultivars via marker-aided selection. Molecular markers are abundant (Staub & Serquen 1996). Therefore, SRAP markers can readily be used to saturate linkage maps of okra in future studies, which would further enhance okra breeding programs. Potential of the other molecular markers that amplify relatively
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less conserved regions of okra genomes, such as intersimple sequence repeats, simple sequence repeats, and amplified-fragment length polymorphism should also be investigated to enhance the okra breeding programs.

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References


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