

IDENTIFICATION OF BANANA ACCESSIONS SAMPLED FROM SUBTROPICAL REGION OF TURKEY USING SRAP MARKERS

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Abstract

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This study was conducted to estimate genetic relationships and natural somatic mutations among selected banana genotypes from different region of Turkey via SRAP molecular markers. Ninety-six banana genotypes including 39 'Dwarf Cavendish', 18 'Grande Nain', 28 'Azman' and 11 other bananas were evaluated. A total of 102 bands were obtained from 19 SRAP primer combinations and 86 (85.5%) of them were polymorphic. Number of bands per primer combination was 5.3 whereas polymorphic bands per primer combinations were 4.5. Genetic similarity of 96 accessions varied between 0.63–1.00. It was determined genetic variation among the accessions which may allow new selections for breeding programs. Considerable variation was also found among genotypes within the same banana cultivar and it was very important for banana breeding and germplasm evaluation. It can be concluded that SRAP markers could readily be used to estimate genetic relationships among banana genotypes and natural somatic mutations.

Key words: molecular identification, *Musa*, mutation

Introduction

Bananas plantains belonging to the *Musa* L. (Family *Musaceae*, order *Gingiberales*) are major food source for millions of people in tropical regions and they are one of the most important fruit crops in the world (Simmonds and Shepherd, 1955). Worldwide, annual banana production is nearly 106 million tons and 10% of this sum was exported, being the second after citrus fruits (FAO, 2011). Some banana types can be cultivated in subtropical regions between 20° and 30° north and south of the equator. Several regions with subtropical climate such as Mediterranean coastline are also suitable for banana culture. In example, edible cultivars were grown on Mediterranean coast lines of Turkey with local microclimate areas for banana production. In this re-

gion, although banana production at open fields goes back to 1930's, protected cultivation was initiated in 1980's in Anamur and Bozyazi towns of Mersin Province and gained significant popularity in 1990's (Gubbuk and Pekmezci, 2001). Turkey's banana production mainly for local consumption rather than export since the production accounts for only the half of total consumption (Pinar et al., 2011). Amount of production increased year by year and reached to 206.000 tons (FAO, 2011).

Although banana breeding programs mostly take place in tropical regions, there are some breeding programs implemented in subtropical regions (Smith et al., 1998). The main goals of banana improvement programs in these subtropical regions are the development of genotypes with higher fruit yield and quality and better adaptatons to cooler cli-

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mates and resistance to pests and diseases. The main climate factors affecting banana production in the cooler subtropics are the greater diurnal temperature fluctuations, lower night temperatures, higher rainfall and stronger winds in summer (Gubbuk et al., 2004).

Banana breeding by conventional strategies is somehow difficult for some edible cultivars because of both male and female sterility and production of parthenocarpic fruits. Mutation breeding and biotechnological methods are desirable tools for the improvement of vegetatively propagated crops (Donini and Sonnino, 1998).

Important commercial banana cultivars within the Cavendish sub-group are triploid, seedless, sterile and parthenocarpic (Khayat et al., 1998). Therefore, banana production has been improved in many countries by either importing promising cultivars/selections from other geographical areas, or *via* the identification of superior and stable local selections (Eckstein et al., 1998; Khayat et al., 1998; Smith et al., 1998). In Turkey, banana production has been made in open fields and greenhouses. In general, new orchards are established with plantlets which obtained from suckers or *via* tissue culture methods. But among the yields (25-80 tons/ha) and qualities of banana orchards there is a great variation (Pinar et al., 2011). These variations are usually caused by somaclonal variations. Therefore, tissue cultured plantlets are especially used in new orchards. It is important to identify superior and stable local selections in subtropical regions for banana breeding programs.

There are many studies on genetic variation and identification of banana accessions and clonal selections. For instance, somaclonal variation has been evaluated at the DNA level in *Musa*. Some of them are random amplified polymorphic DNA (RAPD) (Onguso et al., 2004; Das et al., 2009; Brown et al., 2009), restriction fragment length polymorphism (RFLP) (Bhat et al., 1995), amplified fragment length polymorphism (AFLP) (Ude et al., 2003; Opara et al., 2010), inter simple sequence repeats (ISSR) (Lakshmanan et al., 2007; Lu et al., 2011), simple sequence repeats (SSR) (Mattoos et al., 2010) and sequence-related amplified polymorphism (SRAP) (Phothipan et al., 2005; Youssef et al., 2011).

Sequence-related amplified polymorphism was used firstly in Brassicas for marker development and mapping (Li and Quiros, 2001). For many crops - buffalograsses, *Buchloe dactyloides* Nutt. Engelm (Gulsen et al., 2005), citrus (Uzun et al., 2009) and apricot, *Prunus armeniaca* L. (Uzun et al., 2010a), this system has been used for genetic diversity and fingerprinting studies. The SRAP marker system is a simple and also efficient marker system that can be adapted for a variety of purposes in different crops, including map construction, gene tagging, genomic and cDNA fingerprinting, and

map based cloning. Some advantages of this system were reported as; simplicity, reasonable throughput rate, easy isolation of bands for sequencing and, most importantly, relatedness to open reading frame (ORFs) (Li and Quiros, 2001). Up to now, there is a few reports used SRAP markers to identification of banana germplasm (Phothipan et al., 2005; Youssef et al., 2011).

Although banana has been grown in some part of Turkey since 1934, reports on diversity among banana cultivars and clones are scarce. There is only one report on selection for superior banana phenotypes for breeding programs at Dwarf Cavendish clones (Gubbuk et al., 2004). Therefore, more studies are needed to provide detailed genetic information on bananas grown in Turkey. This study was conducted to estimate genetic relationships and natural somatic mutations among selected banana genotypes from different region of Turkey using SRAP markers.

Materials and Methods

Plant materials

Present study was carried out in Anamur and Bozyazi towns of Mersin Province and Alanya, Gazipasa towns of Antalya Province of Turkey. The first year, 450 greenhouses and 220 open fields were visited for selecting superior types that might be result of natural somatic mutations in 'Dwarf Cavendish', 'Grande Nain' and 'Azman'. Initially, a total of 210 banana clones were selected through observations over 3 years. Then, 96 banana accessions including 39 'Dwarf Cavendish' (D), 18 'Grande Nain' (G), 28 'Azman' (A), two 'Erdemli', one 'Poyo', one 'Williams' and six unknown origin were selected for higher yield (bunch weight, finger number and total weight), quality parameters (bunch and finger lenght) and plant height, an important trait for greenhouse production. The survey was carried out in an area of 4428 ha and 1228 ha of which are in protected cultivation and open field, respectively. These 96 banana accessions were used as material for present study (Table 1).

SRAP analysis

Total genomic DNA was extracted from fresh leaf tissue of 96 banana genotypes for molecular analysis by using the CTAB procedure as described by Doyle and Doyle (1990). DNA concentration was measured with a microplate spectrophotometer (BioTek Instruments, Inc., Vinooski, USA), and 10 ng/mL DNA templates were made using TE (10 mM Tris - HCl, 0.1 mM EDTA, pH 8.0). A total 19 SRAP primers were used for all banana cultivars (Table 2). PCR reaction components and PCR cycling parameters were performed as described by Uzun et al. (2009). PCR products were sepa-

Table 1**List of banana accessions used in this study**

Accession	Cultivar	Selected From	Accession	Cultivar	Selected From
A1	Azman	Alanya	D21	D. Cavendish	Alanya
A2	Azman	Bozyazi	D22	D. Cavendish	Alanya
A3	Azman	Alanya	D23	D. Cavendish	Alanya
A4	Azman	Alanya	D24	D. Cavendish	Gazipasa
A5	Azman	Alanya	D25	D. Cavendish	Anamur
A6	Azman	Anamur	D26	D. Cavendish	Alanya
A7	Azman	Alanya	D27	D. Cavendish	Alanya
A8	Azman	Gazipasa	D28	D. Cavendish	Anamur
A9	Azman	Anamur	D29	D. Cavendish	Anamur
A10	Azman	Anamur	D30	D. Cavendish	Gazipasa
A11	Azman	Anamur	D31	D. Cavendish	Anamur
A12	Azman	Bozyazi	D32	D. Cavendish	Anamur
A13	Azman	Gazipasa	D33	D. Cavendish	Bozyazi
A14	Azman	Anamur	D34	D. Cavendish	Bozyazi
A15	Azman	Anamur	D35	D. Cavendish	Bozyazi
A16	Azman	Alanya	D36	D. Cavendish	Bozyazi
A17	Azman	Anamur	D37	D. Cavendish	Bozyazi
A18	Azman	Bozyazi	D38	D. Cavendish	Anamur
A19	Azman	Anamur	D39	D. Cavendish	Gazipasa
A20	Azman	Gazipasa	ERD4	Erdemli	Erdemli
A21	Azman	Gazipasa	ERD5	Erdemli	Erdemli
A22	Azman	Gazipasa	FR1-1	Unknown	Unknown
A23	Azman	Anamur	FR2-2	Unknown	Unknown
A24	Azman	Anamur	FR-3	Unknown	Unknown
A25	Azman	Erdemli	FR-4	Unknown	Unknown
A26	Azman	Erdemli	G1	Grande Nain	Alanya
A27	Azman	Erdemli	G2	Grande Nain	Anamur
A28	Azman	Erdemli	G3	Grande Nain	Anamur
D1	D.Cavendish	Anamur	G4	Grande Nain	Anamur
D2	D. Cavendish	Alanya	G5	Grande Nain	Anamur
D3	D. Cavendish	Anamur	G6	Grande Nain	Bozyazi
D4	D. Cavendish	Bozyazi	G7	Grande Nain	Anamur
D5	D. Cavendish	Gazipasa	G8	Grande Nain	Bozyazi
D6	D. Cavendish	Alanya	G9	Grande Nain	Anamur
D7	D. Cavendish	Anamur	G10	Grande Nain	Anamur
D8	D. Cavendish	Alanya	G11	Grande Nain	Anamur
D9	D. Cavendish	Alanya	G12	Grande Nain	Anamur
D10	D. Cavendish	Alanya	G13	Grande Nain	Anamur
D11	D. Cavendish	Alanya	G14	Grande Nain	Anamur
D12	D. Cavendish	Anamur	G15	Grande Nain	Bozyazi
D13	D. Cavendish	Gazipasa	G16	Grande Nain	Anamur
D14	D. Cavendish	Alanya	G17	Grande Nain	Anamur
D15	D. Cavendish	Alanya	G18	Grande Nain	Anamur
D16	D. Cavendish	Anamur	KRM-1	Unknown	Unknown
D17	D. Cavendish	Alanya	KRM-2	Unknown	Unknown
D18	D. Cavendish	Gazipasa	PY	Poyo	Anamur
D19	D. Cavendish	Anamur	W	Williams	Anamur
D20	D. Cavendish	Alanya			

Table 2**Total and polymorphic band number and polymorphism ratio of banana accessions**

Primer combinations	Total bands	Polymorphic bands	Polymorphism. %
em1-me1	6	3	50
em2-me1	7	7	100
em3-me2	6	5	83
em3-me3	6	4	67
em3-me4	4	3	75
em3-me5	3	3	100
em4-me3	3	3	100
em6-me11	6	6	100
em6-me2	10	7	70
em6-me3	6	6	100
em7-me10	10	8	80
em8-me4	5	5	100
em8-me8	5	5	100
em10-me7	4	4	100
em11-me6	4	4	100
em12-me9	3	3	100
em14-me3	2	1	50
em14-me4	6	4	67
em16-me3	6	5	83
Mean	5.4	4.5	85.5
Total	102	86	—

rated on 2% agarose gel in 1 x TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 115 volt for 3–5 h. The fragment patterns were photographed under UV light for further analysis. A 100 bp standard DNA ladder was used for SRAP analysis as the molecular standard in order to confirm the appropriate markers.

Statistical analysis

Molecular analysis was carried out as follows: 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 version 2.1 (Rohlf, 2000). A similarity matrix was constructed using SRAP data based on Dice (1945) coefficient. Then, the similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average) to determine genetic relationships among the cultivars studied. The genetic similarity matrix and ultrametric distance matrix produced from UPGMA-based dendrogram with Coph module nested in the same software was compared using Mantel's matrix correspondence test (Mantel, 1967). The result of this test is a cophenetic correlation coefficient r that indicates how well dendrogram represents similarity data. The principal com-

ponents analysis (PCA) of the original binary data matrix was also performed using NTSYS software. The UPGMA and PCA analyses provide alternative approaches and better visualisation of selections on figures.

Results and Discussion

Ninety-six banana selections were evaluated by using SRAP markers. A total of 105 bands were scored from 19 SRAP primer combinations and 89 (85%) of them were polymorphic. Number of bands per primer combination was 5.30 whereas polymorphic bands per primer combinations were 4.5. Similarly, Lu et al. (2011) and Brown et al. (2009) obtained 5–9 bands per primer and about 85% polymorphism ratio based on ISSR and RAPD markers. In another study, Creste et al. (2004) obtained 12.8 fragments per primer from microsatellite markers and Gubbuk and Pekmezci (2001) found 1–10 fragments per RAPD primers. In present study, Em-6-Me2, Em2-Me1 and Em6-Me11 had the highest number of polymorphic bands. The lowest number of polymorphic bands was obtained from Em14-Me3 primers. The highest polymorphism ratio was obtained from Em3-Me4, Em6-Me3, Em2-Me1, Em8-Me4, Em10-Me7, Em11-Me6 and Em8-Me8 primer combinations (100%) whereas the lowest polymorphism ratio was obtained from Em14-Me3 and Em1-Me1 primer combination – 50% (Table 2).

For estimating level of correlation between the similarity matrix and ultrametric distance matrix, initially similarity tree was converted into the ultrametric distance matrix. Then, by using Mantel test, cophenetic correlation between ultrametric similarities of tree and similarity matrix was found to be high ($r = 0.98$, ** $P < 0.01$) suggesting that the obtained cluster strongly represents the similarity matrix.

Genetic similarities of the 96 banana genotypes ranged between 0.63 and 1.00 (Figure 1). Most of the banana genotypes were distinguished and few of them were identical. Two Erdemli bananas, ERD4 and ERD5, were the most distinct genotypes from the others with the similarity value of 0.63. These genotypes had short and thick fruits compared to the other genotypes. They were grown in protected areas of open fields and selected from commercial orchard from Erdemli town of Mersin province. There was no information when these cultivars were brought to Turkey. Most probably the Erdemli cultivar had different origin from the other bananas. Rests of the 96 genotypes were separated into two groups at ~0.85 similarity level. In previous study higher level of genetic variation was found among Southern India bananas (Venkatachalam et al., 2008).

Probably because of they utilized more cultivars for analysis, more variation could be determined. In our den-

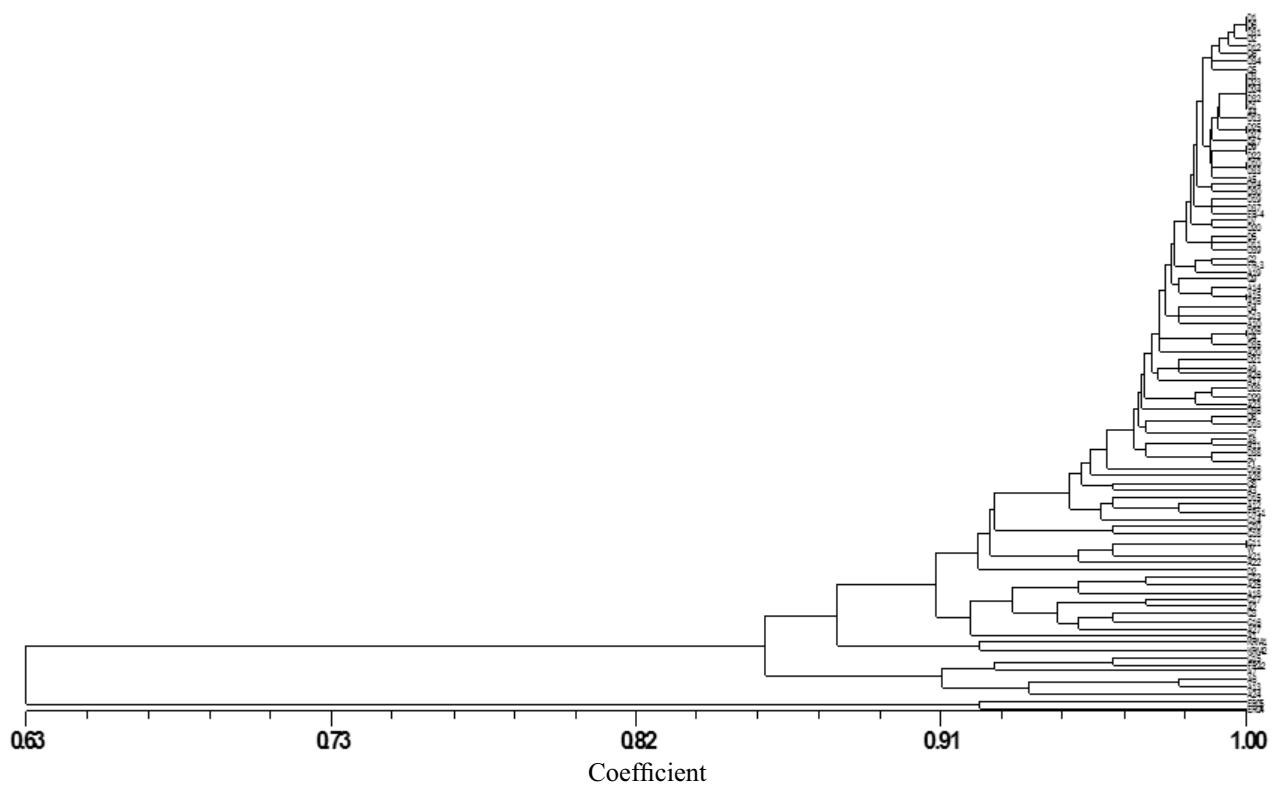


Fig. 1. Dendrogram of the 96 banana accessions based on the 19 SRAP markers

rogram small group contained several Azman genotypes, one Grande Nain and one foreign genotype (FR-2-2). The Grande Nain genotype, G15, in this group was superior with high yield (110 kg bunch weight, data not shown). In the large group, there were many small sub-groups. Two red skin bananas (KRM1 and KRM2) were clustered in the same sub-group. Although there was no clear separation among the cultivars, most of the Dwarf Cavendish genotypes were clustered closely. The lowest genetic variation found in Dwarf Cavendish genotypes. Among the cultivars, Azman clones were the most diverse after Grande Nain. Most of the genotypes showed low level of variation and the genetic similarity was over 0.90. This might be caused by vegetative propagation of these genotypes.

These results were also previously reported for other vegetatively propagated fruit species. The studies indicated low level of diversity in lemon (Uzun et al., 2009), grapefruit (Uzun et al., 2010b) and they assumed that morphological differences in that species were mainly due to mutations. In present study, clones of some banana genotypes were identical. For example G11 ('Grande Nain') and W ('Williams') found to be identical. Although named as 'Williams', morphological characters of this genotype were be-

tween 'Azman' and 'Williams' cultivars (data not shown). Also, D26 ('Dwarf Cavendish') and G4 ('Grande Nain') genotypes were genetically identical. This may be caused by mixed cultivation and wrong nomenclature. On the other hand, this nomenclature may be due to morphological differences and may have been caused by mutations. Similarly, 17 of 116 banana accessions were found as synonym in Indonesian germplasm in previous study (Retnoningsih et al., 2011).

The principal components analysis (PCA) was performed for better visualisation of relations among the accessions studied. The classical PCA is likely an example of dimensionality reduction. Therefore, it is important that the required information is strongly related to the variance in the data (Scholz and Selbig, 2006). The PCA revealed some aspects of interrelations among the studied materials that were not discernable by the cluster analysis (Marak and Laskar, 2010). The results of PCA are demonstrated in Figure 2. PCA-1 and PCA-2 represented 92.8% and 1.9% of the variation in the binary data matrix, respectively. It implies that 94.8% of the total variation in the original dimensions could be represented by just two dimensions defined by the first two PCs. Two-dimensional dispersion showed that two 'Erdemli' genotypes, ERD4 and ERD5, were clearly apart

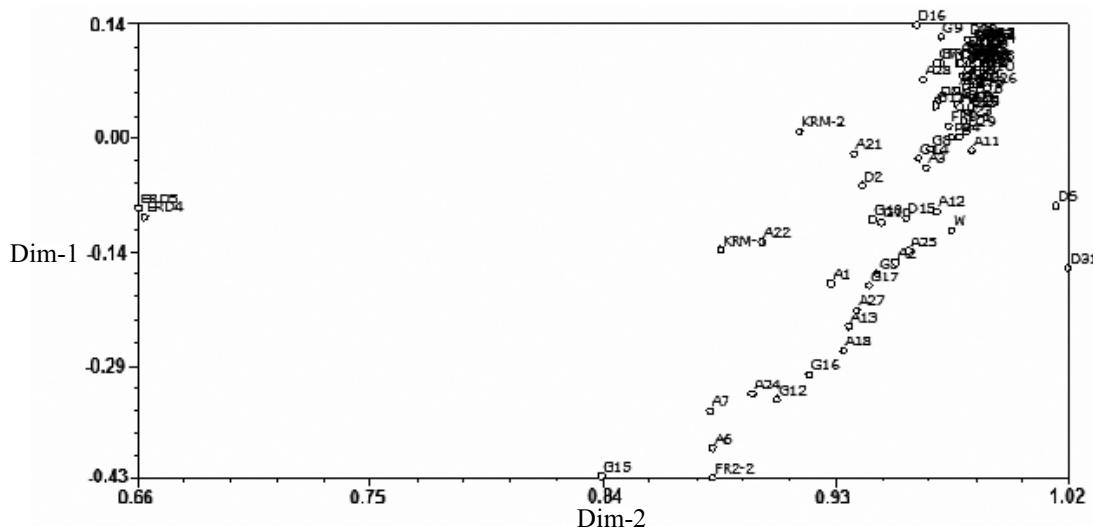


Fig. 2. Principal component analysis using 19 SRAP markers from 96 banana genotypes

from other genotypes. Most of the accessions had low genetic variation.

The use of DNA fingerprinting for *Musa* germplasm conservation and breeding allows identification of species and cultivars and determination of the evolutionary relationships between clones. It also helps identification of duplications among accessions in the field and in tissue culture germplasm banks (Onguso et al., 2004). The present study indicated that there was variation among banana genotypes belong to same cultivar produced in Turkey. This variation was possibly caused by mutation because banana was vegetatively propagated crop. Open field and greenhouse cultivations of banana exist in Turkey and banana is not native. Plantlets are obtained from tissue culture or suckers. So, genetic variation may occur *in vitro* or open field condition. Venkatachalam et al. (2007) determined no variation with molecular markers among eleven *in vitro* micropropagated banana plantlets. They assumed that although genetic change has not been detected, it was possible that some changes might have occurred that go undetected as there is a possibility of point mutations occurring outside of the priming sites. Detected variation in our study was very important for banana breeding and germplasm evaluation. Among the genotypes, there were promising accessions with high yield and low plant height, which may interest banana breeders and producers.

As conclusions, molecular analysis revealed that genetic variation exists among and within banana cultivars. This result may be explained by natural occurred mutations because of greenhouse and open field cultivation in subtropical

condition. Erdemli banana cultivar genetically distincts from others probably caused by different origin. Variation of accessions belonging to same cultivar may offer possibilities of genetic pool for breeders and new cultivars with valuable agronomic traits. SRAP markers was powerful system to estimate genetic relationships among banana genotypes and natural somatic mutations.

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