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Morphological and molecular characterization of banana clones growing in Turkey

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Abstract: This study was conducted to estimate genetic relationships among banana clones growing in Turkey via some morphological parameters and two molecular marker systems. In terms of yield parameters such as bunch weight, hand number, fruit weight, and total fruit number, Grand Nain (GN) clone came to the forefront with the highest values. It was followed by Azman (AZ), Dwarf Cavendish (DC), and Erdemli Yerli (EY) clones, respectively. To see the variation between clones more clearly, 24 RAPD (Random Amplified Polymorphic DNA) primers and 48 SRAP (Sequence Related Amplified Polymorphism) primer combinations were used in molecular analysis. The total number of amplified bands was 194, and 142 of them were polymorphic for RAPD analysis. The total and polymorphic bands per primer ranged from 0 to 14. A total of 272 bands were obtained from SRAP analysis, of which 154 were polymorphic. The total number of bands per primer varied between 0 and 11, and the number of polymorphic bands varied between 0 and 10. According to the dendrogram formed by unweighted pair group method with arithmetic averages (UPGMA) analysis, clones were collected in two main groups. In all evaluation methods, EY was completely separated from the other clones. GN, DC, and AZ were gathered on a single branch in the dendrogram. As a result of the SRAP assessment, DC and GN were the closest related clones. Contrary to the SRAP results, GN and AZ were identified as the most genetically related clones in morphological and RAPD assessments. This study showed that morphological and molecular characterization could be useful to assess the relationship among banana clones.

Key words: Diversity, molecular markers, *Musa*, NTSYS, UPOV

1. Introduction

Banana is an important crop in tropical and subtropical regions, believed to be originated from Indo-China and South-East Asia, where it has many wild species (*Musa acuminata* AA and *M. balbisiana* BB) nowadays (Simmonds, 1959).

Some banana clones can be cultivated in subtropical regions between 20° and 30° north and south of the Equator. In Turkey, the cultivation is only carried out in the Mediterranean climate strip. And most banana growers produce Azman (AZ), Dwarf Cavendish (DC), and Grand Nain (GN) cultivars as cultivation material, and the cultivars have A genome (*M. acuminata*). AZ is thought to be one of the clones of GN mutated over time. However, there is no clear information about AZ. However, it is a commercial variety for the country. In addition to these varieties, there is also Erdemli Yerli (EY) variety that has no commercial importance, but is offered for sale in local markets.

Many phylogenetic studies on the genus *Musa* (Heslop-Harrison and Schwarzacher, 2007; Li et al., 2010; Liu et al., 2010; Nayar, 2010; Christelova et al., 2011; Hřibová et al., 2011; Xavier et al., 2011) demonstrated that none of the five sections of *Musa* previously defined based on morphology was recovered as monophyletic. Over the years, different clones appear in all bananas. In other words, clones that emerge as a result of genetic changes are examples of mutations (Thompson, 2019). Morphological data are very limited in the evaluation of a population, they can be under the influence of environmental conditions; therefore, the genetic potential of the populations cannot be fully determined.

The main objectives of the banana breeding programs in these subtropical regions are to develop genotypes that are better adapted to colder climates with higher fruit yield and quality, and resistant to pests and diseases (Gubbuk et al., 2004). Recently, to identify *Musa* genomes and determine the level of genetic variability between varieties, several techniques have been used via molecular markers, including random amplified polymorphic DNA (RAPD) (Das et al., 2009; Choudhary et al., 2014; Handayani et al., 2018), restriction fragment length polymorphism (RFLP) (Hippolyte et al., 2010), amplified fragment length polymorphism (AFLP) (Opara et al., 2010; Cruz Cardenas

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et al., 2017), sequence related amplified polymorphism (SRAP) (Phothipan et al., 2005; Cruz Cardenas et al., 2017), simple sequence repeat (SSR) (Miller et al., 2010; Nyine at al., 2017; Biswas et al., 2020), inter simple sequence repeat (ISSR) (Choudhary et al., 2014; Silva et al., 2017; Borborah et al., 2020), and conserved DNA-derived polymorphism (CDDP) (Igwe et al., 2021) markers.

Banana has been grown in Turkey since the1930s. However, studies on banana plants are very limited. This is the most detailed study ever done on this subject. This study aims to make the morphological characterization of the variation among some important banana clones grown in Turkey with phenotypic observations to perform molecular characterization using SRAP and RAPD marker systems.

2. Materials and methods

The study was performed on four banana clones (GN, DC, AZ, and EY) located in Mersin city (34°E 36°N, sea level, average annual temperature: 23.3/14.7°C, mean relative humidity: 70%, mean annual precipitation: 138 mm), Erdemli (one of the most important areas of banana cultivation in the country) in Southern Turkey. The materials used in the study were collected taking into account some commercial criteria from different producer greenhouses and open field areas by visiting Erdemli (EY), Anamur (GN), Alanya (DC), and Bozyazı (AZ) districts. They were taken under protection in the greenhouse in Alata Horticultural Research Institute, in 2005. The study was carried out between 2010 and 2011 vegetation years. The average spacing between plants in the greenhouse was 3 m. The horticultural practices included irrigation and fertilization (45 kg of nitrogen, 150 kg of potassium, and 60 kg of phosphate per plant) for a year.

2.1. Morphological characterization

Morphological characterization of each clone was done according to the 45 qualitative and quantitative criteria (such as pseudostem: length, bunch: length, bunch: diameter, fruit: longitudinal ridges, fruit length, fruit: shape of apex, fruit thickness of peel, fruit: color of peel, fruit: color of flesh, fruit: firmness of flesh) of International Union for the Protection of New Varieties of Plants (UPOV, 2010). Selected plants were flowered in June, July, and August, and fruit bunches were harvested in October, November, and December.

2.2. Molecular characterization

Total genomic DNA was extracted from fresh leaf tissues using the CTAB method as described by Pancholi (1995). Before reading in the spectrophotometer (Shimadzu UV-160A), it was determined whether they contain RNA, protein, and phenol in the DNAs of different banana clones, as well as whether there are breaks in the DNAs by running the extracted DNA in a gel.

A total of 24 RAPD primers and 48 SRAP primer combinations were used for all banana clones. The primer names and sequences were given in Table 1. Polymerase chain reaction (PCR) of RAPD was performed according to Pancholi (1995). In detecting variations between clones; 25 ng genomic DNA, 2.5 mM $MgCl_2$, 1.25 Unit Taq polymerase, 10 µM Primer, 0.4 mM of each dNTPs (dATP, dCTP, dGTP and dTTP) and 50 mM KCl, 1 mM Tris-HCl (pH:9) and 1X PCR buffer were used. PCR conditions: denaturation at 94 °C for 2.30 min, annealing at 35 °C for 1.30 min, extension at 72 °C for 2.00 min and 1 cycle; denaturation at 94 °C for 2.30 min, annealing at 35 °C for 1.30 min, extension at 72 °C for 2.00 min and 44 cycles; extension at 72 °C for 10.00 min and 1 cycle. 0.8% agarose gel was prepared. 1X TAE buffer was used in agarose gel preparation and gel run. DNA samples were run at 50 volts for 1.5 h by electrophoresis method. After keeping, the gel was visualized in the transliminator under an UV lamp.

Polymerase chain reaction (PCR) of SRAP was performed according to Uzun et al. (2009). Each of 15 µL reaction consisted of 1.33 mM of primers, 200 mM of each dNTP, 1.5 µL of 10 PCR Buffer, 2 mM of $MgCl_2$, 0.8 mg/mL Bovine serum albumin, 5.8 mL ddH_2O, 1 unit of Taq polymerase and 20 ng of DNA template. PCR conditions; denaturing at 94 °C for 2 min, five cycles of three steps: 1 min of denaturing at 94°C, 1 min of annealing at 35 °C, and 1 min of elongation at 72 °C. In the following 35 cycles, the annealing temperature was increased to 50 °C, and for extension, one cycle 5 min at 72 °C. Amplification products of SRAP analysis were resolved by electrophoresis on 1.5% agarose gels in 1X TAE buffer and stained with ethidium bromide at 115 V for 3.5 h and visualized on an UV transilluminator.

2.3. Data analysis

Morphological data were presented as mean ± SD and subjected to two-way ANOVA with randomized plot design for each parameter using JPM 5.0.1. software (SAS Institute, Cary, NC, 1989) followed by the LSD test ($p < 0.05$).

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 (Rohlf, 1998). The genetic similarity matrix was calculated using the coefficients of Nei and Li (1979). Cluster analysis was conducted based on the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) using NTSYS-pc version 2.0 software (Rohlf, 1998).

3. Results and discussion

3.1. Evaluation of plant features

The pseudostem of banana was the result of the growth and development of the leaf midrib surrounding the rhizome

Table 1. RAPD and SRAP primers and sequences used in the study.

RAPD primers	Sequence (5'-3')	SRAP primers	Sequence (5'-3')
OPH02	TCGGACGTGA	EM1	GAC TGC GTA CGA ATT AAT
OPP19	GGGAAGGACA	EM2	GAC TGC GTA CGA ATT TGC
OPY6	AAGGCTCACC	EM3	GAC TGC GTA CGA ATT GAC
OPAH16	CAAGGTGGGT	EM4	GAC TGC GTA CGA ATT TGA
TIBMBDO7	GAGCTGGTCC	EM6	GAC TGC GTA CGA ATT GCA
TIBMBB03	TCACGTGGCT	EM7	GAC TGC GTA CGA ATT CAA
OPR1	TGCGGGTCCT	EM8	GACTGCGTACGAATTGGT
TIBMBD17	GTTCGCTCCC	EM9	GACTGCGTACGAATTCGG
OPAH19	GGCAGTTCTC	EM10	GACTGCGTACGAATTCAG
OPAH2	CACTTCCGCT	EM11	GACTGCGTACGAATTCCA
TIBMBA03	GTGCGAGAAC	EM12	GACTGCGTACGAATTCTC
OPH02	TCGGACGTGA	ME1	TGA GTC CAA ACC GGA TA
OPAD11	CAATCGGGTC	ME2	TGA GTC CAA ACC GGA GC
OPAI08	AAGCCCCCCA	ME3	TGA GTC CAA ACC GGA AT
TIBMBB13	CTTCGGTGTG	ME4	TGA GTC CAA ACC GGA CC
TIBMBB07	GAAGGCTGGG	ME5	TGA GTC CAA ACC GGA AG
OPAC12	GGCGAGTGTG	ME6	TGA GTC CAA ACC GGA CA
OPA13	CAGCACCCAC	ME7	TGA GTC CAA ACC GGA CC
TIBMBB09	AGGCCGGTCA	ME8	TGA GTC CAA ACC GGA GC
TIBMBL08	TGCGGGTTCC	ME9	TGAGTCCAAACCGGTGT
TIBMBA07	GGGTCGCATC	ME10	TGAGTCCAAACCGGTCA
OPAD04	GTAGGCCTCA		
OPY13	GGGTCTCGGT		
TIBMC08	GGTCTTCCCT		

(Sumardi and Wulandari, 2010). The highest pseudostem height was found in AZ (362.00 cm) whereas the shortest was observed in DC (261.00 cm) (Table 2). Mattos et al. (2010) investigated variable plant agronomic (the number of fruits and hands, etc.) and yield characteristics of 26 banana accessions including wild diploid and improved, triploid, and tetraploid genotypes. They determined pseudostem heights between 144.00 and 354.00 cm. Ara et al. (2011) also revealed pseudostem heights between 167.00 and 319.00 cm among banana cultivars/lines. These findings supported our results.

Considerable variation in height, color, and disposition of the pseudostem occurs and is used to distinguish banana cultivars (Karamura et al., 2011). When grouped as tapering of pseudostem length, EY was weak, AZ and GN were medium, and DC was strong. The plant growth habit, compactness of crown and overlapping of leaf sheaths can be regarded as important criteria in adjusting the planting distance. The banana cultivars with genome groups AA, AAA, AAB, ABB, and BB share similar characteristics including normal dwarfism (leaves not overlapped and leaf ratio superior to 2.5) (Wahyudi and Rifliyah, 2020). In our study, DC showed a compact crown while AZ, EY, and GN exhibited a loose crown. The plant growth habit and overlapping of leaf sheaths were upright and weak in EY, spreading and medium in GN, upright and medium in AZ, and compact and strong in DC, respectively. When we evaluated the plants according to the pseudostem color and intensity of anthocyanin coloration, EY was found greenish-yellow and weak, but others were reddish-green and medium (Table 3).

The cultivars used in the study were grown on the Mediterranean coastline. Purseglove (1972) stated that pseudostem height varied across cultivars and agro-ecological conditions and from 4 m on the plains to 8 m in sheltered valleys for the AAA cultivar 'Gros Michel'. Likewise, Cavendish clones were found to be relatively tall in lowland areas where conditions are ideal but shorter at

Table 2. Mean and standard deviation values for the quantitative parameters of pseudostem, leaf, and female flower.

Parameters	Clones			
	AZ	DC	EY	GN
Pseudostem height (cm)	362.00^{a} ± 28.78	261.00^{c} ± 32.08	328.00^{abc} ± 6.07	307.00^{b} ± 39.80
Pseudostem diameter (cm)	86.51 ± 9.17	91.60 ± 8.18	75.79 ± 4.9	90.70 ± 6.94
Leaf blade length (cm)	266.80^{a} ± 5.95	199.93^{c} ± 5.16	183.18^{d} ± 2.70	243.56^{b} ± 3.22
Leaf blade width (cm)	105.76^{b} ± 4.33	97.39^{c} ± 2.68	75.20^{d} ± 5.61	110.60^{a} ± 4.22
Leaf blade length/width ratio	2.52^{a} ± 0.10	2.05^{c} ± 0.72	2.44^{a} ± 0.18	2.17^{b} ± 0.10
Female flowering length (cm)	202.49^{a} ± 2.03	171.40^{b} ± 4.49	116.21^{c} ± 3.92	123.34^{c} ± 2.17
Female flowering width (cm)	51.45^{a} ± 1.69	41.24^{c} ± 0.91	41.64^{bc} ± 1.65	43.49^{b} ± 2.40
Female flowering length/width ratio	3.93^{b} ± 0.12	4.15^{a} ± 0.15	2.79^{c} ± 0.17	2.83^{c} ± 0.16

*Data are the mean ± SDA. Values represent the means of ten independent biological replicates. Lettering is valid for the same line. Significant differences between means are shown by different letters ($p \leq 0.05$)

Table 3. Qualitative parameters of the studied clones.

Parameters	AZ	DC	EY	GN
Bunch: attitude of fruits	Strongly turned up	Moderately turned up	Strongly turned up	Moderately turned up
Bunch: compactness	Medium	Compact	Medium	Compact
Bunch: shape	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Fruit shape of apex	Truncate	Bottle-necked	Bottle-necked	Truncate
Fruit: color of peel (a.m.)	Greenish yellow	Medium yellow	Greenish yellow	Medium yellow
Fruit: longitudinal curvature	Evenly curved	Evenly curved	Straight	Evenly curved
Fruit: persistence of floral organs	Present	Present	Absent	Present
Plant: growth habit	Upright	Drooping	Upright	Spreading
Leaf blade: color of midrib on lower side	Green	Green	Green	Green
Leaf blade: shape of base	Both sides acute	Both sides rounded	Both sides rounded	One side rounded and one side acute
Rachis: persistence of hermaphrodite flowers	Present	Present	Absent	Absent
Male inflorescence: persistence	Present	Present	Present	Present
Male inflorescence: shape (in cross section)	Medium ovate	Broad ovate	Medium ovate	Broad ovate
Male inflorescence: shape of apex of bract	Broad acute	Broad acute	Obtuse	Right angle
Plant: compactness of crown	Loose	Compact	Loose	Loose
Male inflorescence: overlap of bracts	Medium	Medium	Strong	Weak
Pseudostem overlapping of leaf sheats	Medium	Strong	Weak	Medium
Pseudostem: color	Reddish green	Reddish green	Greenish yellow	Reddish green
Pseudostem: intensity of anthocyanin coloration	Medium	Medium	Weak	Medium
Pseudostem: tapering along lenght	Medium	Strong	Absent-weak	Medium
Rachis: persistence of bracts	Strong	Strong	Absent-weak	Absent-weak
Rachis: prominence of scars	Strong	Strong	Strong	Weak

higher altitudes (Stover and Simmonds, 1987). Previous studies also indicated that there was a great variation among banana cultivars/lines for most of the agronomic and yield traits. For example, Pinar et al. (2020) reported that for Dwarf Cavendish, Azman, and Grand Nain genotypes grown in greenhouses, stem heights (SH) varied between 202.00 and 300.00 cm, 280.00 and 450.00 cm, and 300.00 and 450.00 cm, respectively. The means of their result were higher in Dwarf Cavendish and Grand Nain and lower in Azman than our results. Mattos et al. (2010) used 26 banana accessions including wild diploid and improved, triploid, and tetraploid genotypes. The plant height ranged from 144.00 cm for the triploid Walha (AAB genome) to 354.00 cm for tetraploid hybrid Ambrosia (AAAA genome), with a mean of 279.00 cm. Results indicated wide genetic variability for plant height among the accessions tested. All clones used in the study were triploid and compared to this study it was understood that there was no direct relationship between plant height and ploidy level (Pinar et al, 2015a).

Banana leaves are light green in color, smooth, and glossy and attain a very large size, often being used as a temporary shade for other crops (Karamura et al., 2011). Leaf retention is affected by prevailing soil fertility and soil moisture levels. Air temperature, day length, plantation age, plant density, and plant stature are also known to influence leaf emergence, notably in the Cavendish and Gros Michel subgroups (Allen et al., 1988). A total of 22 qualitative and 23 quantitative characters were recorded and evaluated to establish the variability among the studied clones and 5 of them were about leaf morphology. Some vegetative characters can be used as an indicator to determine ploidy levels of banana genotypes and one of the most important ones is leaf morphology (Pascua and Espino, 1987). According to the observations on the leaf, the shape of the leaf blade base in GN was determined as one side rounded and one side acute, DC and EY were as both sides rounded. However, AZ was both sides acute. The leaf blade: the color of midrib on the lower side of all cultivars was green (Table 3).

The first few leaves of banana plants are essentially bladeless. Therefore, the size of the lamina increases in both dimensions with each succeeding leaf tending to exceed its predecessor (Barker, 1968). For leaf blade measurements in the study, after the total number of leaves was determined, measurements were made on the median leaf. Although the lowest leaf blade length and width were registered by EY, the highest of that registered by AZ and GN, respectively (Table 2). Balkic et al. (2016), on Dwarf Cavendish banana cultivar, reported that the plants had the highest bunch and fruit weight when the male flowers were cut after the female flowers dried. In our study, DC had hermaphrodite flowers. However, GN, AZ, and EY did not have them. This result will be considered especially in crossing studies.

Male inflorescences have the potential to be used as explants for rapid micropropagation of *Musa* spp. (Darvari et al., 2012). DC exhibited persistence of male inflorescence compared to the others. While AZ and EY had a medium ovate male inflorescence shape, DC and GN had a broad ovate. Bract shape and its opening are very important in the supply of male flowers for breeding studies. AZ and DC had a broad acute bract apex, while GN and EY had a right angle and obtuse bract apex, respectively (Table 3).

Typology of bract scars was also a distinctive character among the genome group of the banana cultivars. Banana cultivars derived from *M. acuminata* have prominent bract scar while banana cultivars derived from *M. balbisiana* have scarcely prominent bract scars (Wahyudi and Rifliyah, 2020). In the study, GN had a weak prominence of scar, while the others had a strong prominence of the scar. The persistence of bracts was found weak in GN and EY but strong in AZ and DC (Table 3). Considerable variation was observed in the evaluated plant materials as a parthenocarpic fruit where banana is only formed with female flowers. However, hermaphrodite and male flowers are located on the bunch. In many varieties, male flowers open reflexively, but they are shedded later (Karamura and Karamura, 1995).

The female flowering length was very short in EY with 116.21 cm and very long in AZ with 202.49 cm. While the female flowering width was determined, the largest in AZ with 51.45 cm, the smallest was determined in DC with 41.24 cm. The female flowering length/width was changed from 2.79 to 4.15 (Table 2). Irrespective of the endogenous mechanism which controls femaleness or maleness of the flower, this process is influenced by environmental conditions preceding inflorescence emergence (Turner, 1970). Smirin (1960) indicated that low temperatures reduced bunch size, which is a function of the number of female flowers. EY and DC are usually grown in open fields in Turkey. For this reason, the female flowering length and width may be shorter than others besides the genetic reasons.

3.2. Evaluation of bunch features

The bunch characteristics vary among banana cultivars (Al-Hosni et al., 2010). The bunch dimensions are important in designing packaging for bulk transportation of whole banana bunches (Wills et al., 1989). The bunches reaching harvest maturity were cut by measuring 5 cm above the first hand. The shape of the bunch was also found conical in all clones (Table 4). The highest and lowest bunch weights were reported by GN (36.40 kg) and EY (9.06 kg), respectively (Table 4). In Philippines, the bunch weights were between 6.30 kg (Rose) and 46.10 kg (FHIA-17) (Gervacio et al., 2008); in Egypt, bunch weight and fruit

number of Williams were 26.00 kg and 11.0, respectively (Barakat et al., 2011); in Nigeria, bunch weights and the number of fruits were 8.50–15.90 kg and 6.0 to 18.0, respectively (Adebayo et al., 2009). The bunch weight and the number of fruits obtained from the banana clones used in our study showed a higher average value than Williams banana variety. Measurements made in four banana clones were found to be higher than all values obtained from these studies (bunch weights and number of fruits).

Growth of the inflorescence stalk is rapid and the hands become separated by several centimeters of the stalk. The length of the bunch stalk was determined very short in DC (45.82 cm) and very long in GN (92.49 cm) (Table 4). Mattos et al. (2010) revealed a bunch stalk length between 14.67 cm and 70 cm among 26 banana accessions cultivars/lines which were lower than the current study.

The bunch length is the distance of the points where the first-hand starts and the last hand ends. Regarding the length of the bunch, the maximum value was 125.20 cm (GN), and the minimum value was 93.88 cm (DC). The diameter of the middle point of the bunch is determined as very narrow in EY (41.28 cm) and very broad in AZ (53.52 cm). The bunch length/width ratio was varied between 1.90 and 2.74 (Table 4). GN and DC showed a compact bunch while AZ and EY showed a medium compact bunch. The attitude of fruits on bunch was observed horizontal to slightly turned up in GN, DC, and AZ moderately turned up in EY (Table 5). After the bunch has been harvested, the distance between the upper and lower point of the bunch is bunch width. The bunch distance of hands was found very short in EY (9.49 cm) whereas it was found very long in DC (15.30 cm).

Table 4. Mean and standard deviation values for the quantitative parameters of bunch.

Parameters	Clones			
	AZ	DC	EY	GN
Bunch stalk length (cm)	$72.85^{b} \pm 1.74$	$45.82^{d} \pm 1.63$	$61.76^{c} \pm 1.91$	$92.49^{a} \pm 2.80$
Bunch weight (kg)	$34.48^{a} \pm 6.05$	$29.50^{b} \pm 4.65$	$9.06^{c} \pm 0.91$	$36.40^{a} \pm 3.66$
Bunch length (cm)	$101.75^{b} \pm 2.12$	$93.88^{c} \pm 2.61$	$82.30^{d} \pm 3.40$	$125.20^{a} \pm 3.60$
Bunch width (cm)	$53.52^{b} \pm 2.51$	$42.13^{c} \pm 2.14$	$41.28b^{c} \pm 1.75$	$45.58^{a} \pm 2.28$
Bunch length/width ratio	$1.90^{c} \pm 0.09$	$2.22^{b} \pm 0.07$	$1.99^{bc} \pm 0.11$	$2.74^{a} \pm 0.13$
Bunch distance of hands (cm)	$11.03^{b} \pm 0.60$	$15.30^{a} \pm 1.35$	$7.47^{c} \pm 0.98$	$9.49^{c} \pm 1.07$

*Data are the mean ± SDA. Values represent the means of ten independent biological replicates. Lettering is valid for the same line. Significant differences between means are shown by different letters ($p \leq 0.05$)

Table 5. Mean and standard deviation values for the quantitative parameters of fruit.

Parameters	Clones			
	AZ	DC	EY	GN
Fruit number (n)	$26.00^{a} \pm 1.62$	$23.00^{b} \pm 1.49$	$14.00^{c} \pm 1.13$	$25.00^{a} \pm 1.71$
Hand number (n)	11.50 ± 1.08	12.20 ± 1.47	9.10 ± 0.87	12.35 ± 0.94
Fruit weight (g)	$111.86^{a} \pm 9.22$	$94.20^{b} \pm 5.75$	$82.70^{c} \pm 15.38$	$114.15^{a} \pm 9.38$
Total fruit number (n)	$287.00^{a} \pm 32.98$	$276.09^{a} \pm 39.60$	$122.60^{b} \pm 14.43$	$303.12^{a} \pm 27.45$
Fruit thickness of peel (mm)	$3.04^{a} \pm 3.39$	$2.60^{b} \pm 2.69$	$3.55^{a} \pm 3.65$	$2.29^{c} \pm 2.49$
Fruit length (mm)	$18.88^{a} \pm 0.81$	$17.31^{b} \pm 1.38$	$15.47^{b} \pm 1.26$	$19.54^{a} \pm 0.93$
Fruit width (mm)	$11.98^{a} \pm 0.91$	$11.98^{a} \pm 0.96$	$11.74^{ab} \pm 0.65$	$10.79^{b} \pm 0.88$
Fruit length/width ratio	$1.58^{c} \pm 0.15$	$2.73^{a} \pm 0.29$	$1.31^{bc} \pm 0.09$	$1.81^{b} \pm 0.18$

*Data are the mean ± SDA. Values represent the means of ten independent biological replicates. Lettering is valid for the same line. Significant differences between means are shown by different letters ($p \leq 0.05$)

3.3. Evaluation of fruit features

The physical size of fruits is useful in designing processing (Owolarafe and Shotonde, 2004), and especially the data on fruit size are important in the design of classification equipment in the banana industry (Wasala et al., 2012). Furthermore, the fruit length has been used to assess the maturity of the bunch before harvest (Dadzie and Orchard, 1997). GN had noticeably greater fruit length (19.54 mm), fruit weight (114.15 g), and total fruit number (303.12). However, EY had the smallest values. Unlike the fruit length results, the highest fruit width was determined in AZ and DC and very narrow in GN. The fruit length/width ratio was changed from 0.09 to 2.73 (Table 5). Salunke (1984) reported that the fruit weight at the proper stage of maturity of bananas from the Cavendish group was 133.00–140.00 g and the length was 16.3–17.7 cm. In the characterization of Embul, Seeni, and Kolikuttu local cultivars by Wasala et al. (2012), it was reported that average fruit lengths were 10.5, 10.5, and 14.3 cm, respectively. Our results indicated a wide genetic variability compared to the above results. The length of the pedicel varied from 3.50 cm to 4.67 cm; the highest value was found in AZ and the lowest of that was found in EY. The fruits of EY showed the highest fruit thickness of peel (3.55 mm), followed by AZ (3.04 mm), and the lowest value was recorded by GN (2.29 mm) (Table 5). Kachru et al. (1995) reported that green fruit peel thickness was 3.65 mm and 2.95 mm in cultivars Dwarf Cavendish and Nendran, respectively. The peel thickness of Grand Nain, Kalyani, Poyo, Nendran, Cooking 1, and Champa cultivars was reported by Kuchi et al. (2017). They were changed between 0.29 and 0.46 cm. The results are in accordance with our findings. Tak et al. (2015) reported that the fruit pedicel length in Grand Nain cultivar was 2.25 cm. In another study conducted on the Saba cultivar, Gueco et al. (2020) stated that the fruit pedicel length and peel thickness were 26.7 mm and 2.9 mm, respectively. Results indicate that our clones have shown a long fruit pedicel.

The number of fruits on the third hand was very low in EY (14.0) and very high in AZ (26.0) (Table 5). In the cultivation of bananas, the fruits on the bunch differ in size and it is reported that the fruits at the end of the bunch are 30%–40% smaller than the fruits in the upper parts of the bunch and this is caused by a developmental delay between the fruits (Jullien et al., 2001). Therefore, when calculating the fruit weight, fruit length, and diameter, the arithmetic means of three fruits taken from the middle of the third hand of each clone were taken into consideration. The number of hands on the bunch was found to be very high in GN (12.35) whereas it was very low in EY (9.10) (Table 5). Gubbuk et al. (2004) reported that the hand numbers of different types of Dwarf Cavendish were determined as 10.6 in the open field and 12.9 under greenhouse. Pereira et al. (2000) observed that the average plantation produced 17.7 kg bunch weight and 9.1 hands. Khalequzzaman et al. (2009) also determined some morphological banana features like bunch length (87.90 cm), bunch weight with peduncle (25.81 kg), peduncle weight (1.83 kg), hand weight (23.98 kg), weight per hand (2.67 kg), fingers per bunch (158.20), fingers per hand (17.58), and length per finger (19.98 mm). In our study, bunch weight was measured together with peduncle. When these results were compared, ours were almost in agreement with their results. Njuguna et al. (2008) reported that fruit length, fruit diameter, and finger length/diameter of eight banana varieties varied as 18.30–24.70 cm, 11.8–13.9 cm, and 1.3–1.9, respectively. Similar results were reported by Lima et al. (2005), who assessed triploid and tetraploid banana genotypes and found a variation in fruit length of 13–18 cm. In our study, the length of the fruit was 15.47–19.40 cm, fruit diameter was 10.79–11.98 cm, and finger length/width ratio was 1.31–2.73. These results were consistent with the previous results. Javed et al. (2002) reported that the weight of the bunch was 1.96–9.86 kg, the number of hands was 4.9–10.0, the finger length was 6.92–14.94 cm, and the finger diameter was 1.44–3.50 cm in 14 genotypes in Malaysia. When the clones used in our experiment are compared to these results, the bunch weight of the EY was lower but other clones had higher values. Pinar et al. (2020) evaluated some parameters such as fruit length, fruit weight, number of hands, number of fingers in their study in the greenhouse and open field. Our clones had partially higher values than the plants grown in the open field and lower than the greenhouse.

The breaking point of fruit from the hand, midpoint, and tip are the properties that constitute morphological structure and size. It was reported that these morphological features can be used to distinguish and characterize the cultivars (Dadzie and Orchard, 1997). In our experiment, the fruit shape of the apex was determined as truncate in DC and pointed in GN, AZ, and EY. An important parameter is used to distinguish fruit curvature. Long-fingered banana fruits are preferred more compared to short ones in all uses (Karamura and Karamura, 1995), but fruit lengths vary due to bending in fruit during measurements. It was determined as medium in GN, DC, and AZ, and as shorter in EY. After fruit ripening, the fruit peel color was observed as medium yellow in GN and DC, and dark yellow in AZ and EY. The persistence of floral organs on fruit was only observed in EY. The similarity coefficients were determined using morphological traits. It was changed from –0.1221 to 0.3766.

The similarity matrix was then employed in the construction of a dendrogram via UPGMA. Clones were divided into two groups. GN and AZ showed a similarity of 0.3766 among the four clones assessed in this study. GN,

DC, and AZ were collected on a branch alone, and DC was left as a single branch, and it was separated from AZ and GN. The foreign origin of EY banana clone was separated from the others and determined as an outgroup (Figure 1). Consequently, diversity analysis based on morphological traits could distinguish banana clones in accordance with their genetic backgrounds. Similarly, Pinar et al. (2020) reported that the closest genetic similarities were observed in clones of Grand Nain and Azman, and Erdemli Yerli was the most distinct one which supports our findings.

3.4. Evaluation of molecular data

Four banana clones were assessed concerning genetic diversity by RAPD analysis. The total number of amplified bands was 194, and 142 of them were polymorphic. The total and polymorphic bands per primer ranged from 0 to 14. In terms of the number of polymorphic bands, the primer TIBMBA07 produced the lowest number of bands (0) while primers TIBMBL08 and OPH02 gave the highest number of bands (14). The mean polymorphism rate of the RAPD primers was 73.19% (Table 6).

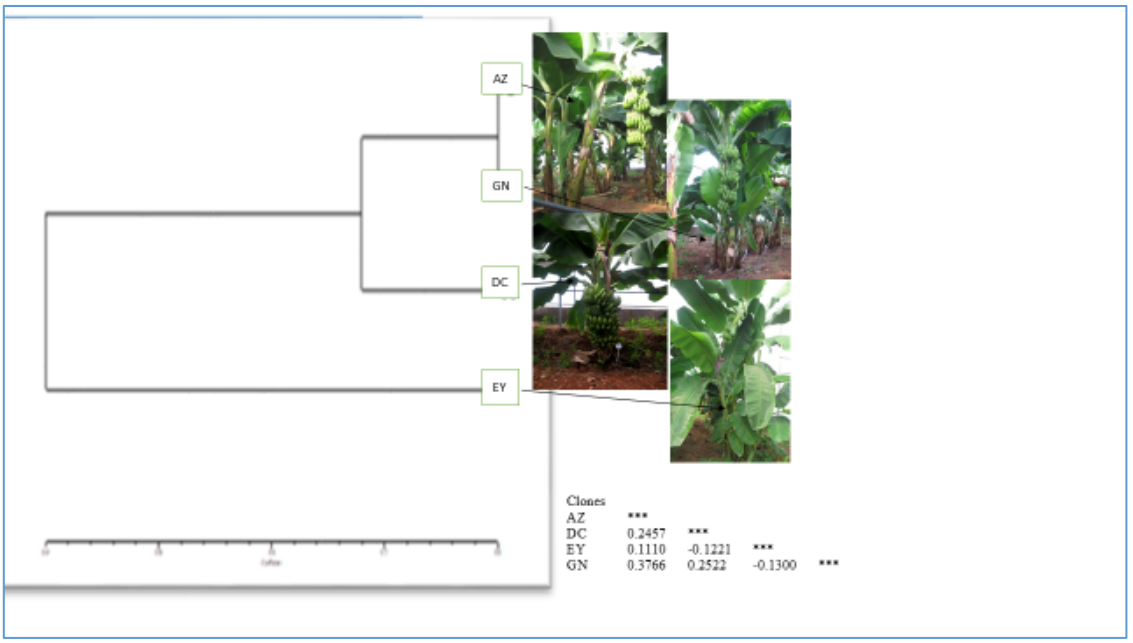

Figure 1. Clustering of four banana clones based on morphological data and similarity matrix values.

Table 6. RAPD primers on four banana clones investigated. TB: total bands; NPB: number of polymorphic bands; P: polymorphism (%).

Primers	TB	NPB	P	Primers	TB	NPB	P
OPH02	14	14	100.00	OPAD11	5	2	40.00
OPP19	11	11	100.00	OPAI08	5	3	60.00
OPY6	4	3	75.00	TIBMBB13	6	6	100.00
OPAH16	7	2	28.57	TIBMBB07	10	9	90.00
TIBMBDO7	7	2	28.57	OPAC12	10	5	50.00
TIBMBB03	6	2	33.33	OPA13	13	10	92.30
OPR1	8	7	87.50	TIBMBB09	13	10	76.92
TIBMBD17	6	6	100.00	TIBMBL08	14	14	100.00
OPAH19	4	2	50.00	TIBMBA07	5	0	0.00
OPAH2	8	7	87.50	OPAD04	8	6	75.00
TIBMBA03	7	4	57.14	OPY13	9	7	77.77
OPHO2	6	4	66.66	TIBMC08	8	6	75.00

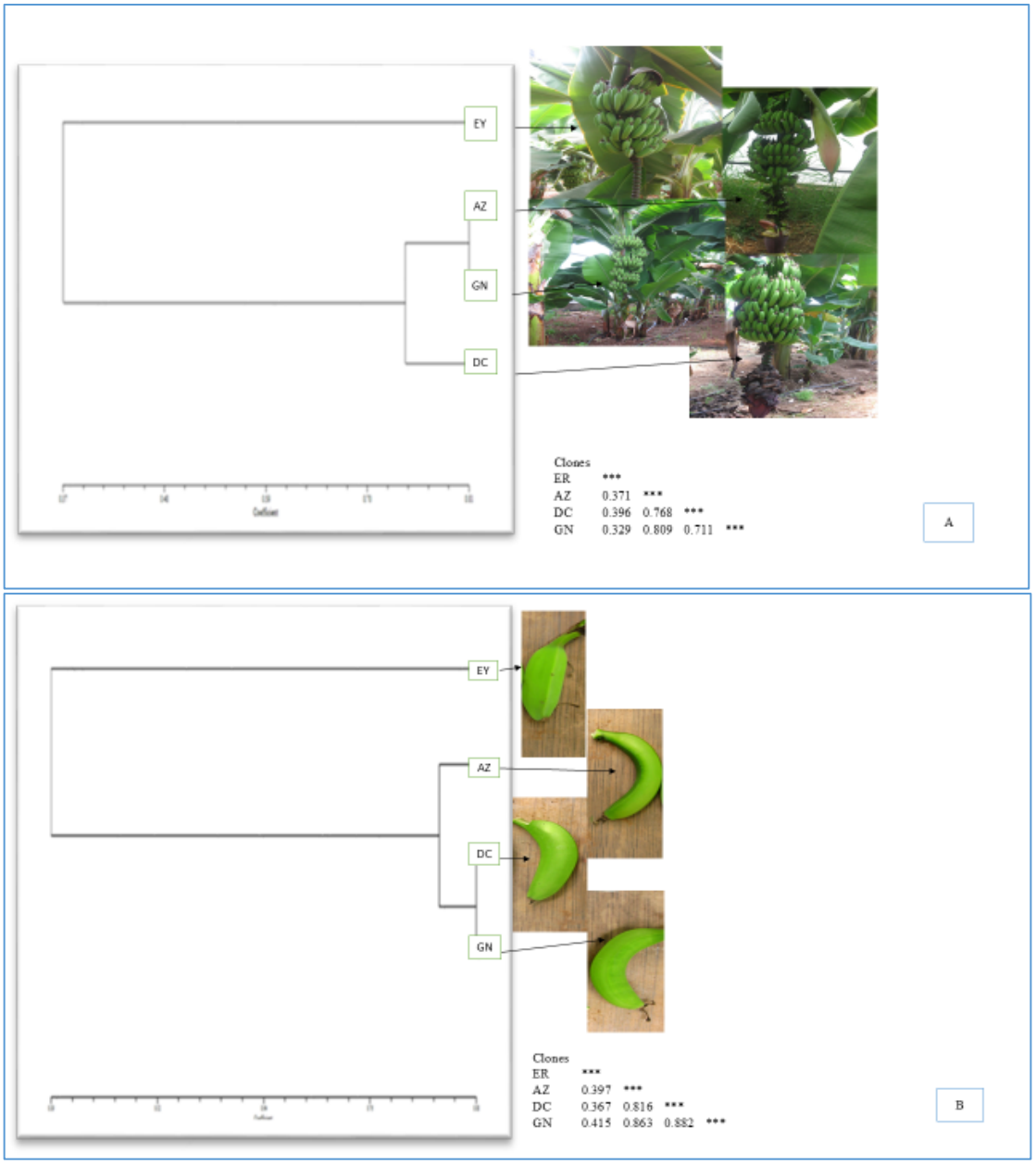

Figure 2. Clustering of four banana cultivars based on molecular data and similarity matrix values. A: RAPD, B: SRAP.

RAPD analysis has also been used to detect variation in gamma-irradiation induced mutants of the Cavendish cv. Grand Naine (Kaemmer et al., 1992) and micropropagated New Guinea Cavendish and Williams cultivars (Damasco et al., 1996). Crouch et al. (2000) identified only a weak relationship between RAPD-based genetic and phenotypic similarities in a study involving 76 plantain landraces. However, Engelborghs et al. (1999) found a significant correlation between molecular diversity and morphotype grouping. Pillay et al. (2001) reported that the highland bananas are closely related with a narrow genetic base. There were sufficient RAPD polymorphisms that were collectively useful in distinguishing the cultivars. The results of the present study demonstrate that RAPD analysis can be used to detect genetic variation in bananas. When the dendrogram formed by UPGMA analysis using RAPD data of 4 clones and using the Nei and Li (1979) similarity coefficients were examined, it was seen that the clones were divided into two groups, as the main group and a small group. The large main group was again divided into two groups within itself. Two small main groups were divided into two branches. The foreign origin EY clone

was separated from the others and determined as an outgroup. GN, DC, and AZ were gathered in a single branch. DC formed a single branch and separated from AZ and GN that were seen as the most closely related clones (Figure 2A).

There are indications that retrotransposons are responsible for spontaneous mutations in plants (Hirochika, 1997). AZ is thought to be one of the clones of GN mutated over time. However, there is no clear information about AZ. As seen in Figure 2A, the highest similarity rate among the clones was found between AZ and GN with 0.809. The lowest similarity was recorded by EY and GN (0.329). Parallel to our study, Pinar et al. (2020) reported that Azman and Grand Nain were genetically closely related clones.

RAPD primers do not anneal to areas of the genome responsible for the morphological variation resulting in nonrandom sampling of the genome, having an insufficient number of polymorphisms (Pillay et al., 2000). To see the variation between clones more clearly, 48 SRAP primer combinations were evaluated as well as RAPD primers. A total of 272 bands were obtained, of which 154 were polymorphic. The total number of bands per primer varied between 0 and 11, and the number of polymorphic bands varied between 0 and 10. In terms of the number of polymorphic bands, the primer combinations Me1xEm1, Me2xEm6, Me10xEm6, Me10xEm11, and Me10xEm12 produced the lowest (0) bands while the primer combination Me3xEm6 produced the most (10) bands. The mean polymorphism rate of 48 SRAP primer combinations used in the study was found to be 56.61% (Table 7).

According to the dendrogram formed by UPGMA analysis in SRAP, clones were collected in two main groups. Similar to the RAPD results, EY was completely separated from the other clones. GN, DC, and AZ were gathered on a single branch. Contrary to the RAPD results, GN and DC were identified as the most genetically related clones.

Table 7. 48 SRAP marker combinations on four banana clones investigated. TB: total bands; NPB: number of polymorphic bands; P: polymorphism (%).

Primers	TB	NPB	P	Primers	TB	NPB	P
Me9xEm1	1	1	100.00	Me1xEm1	2	0	0.00
Me9xEm2	4	2	50.00	Me1xEm3	6	1	16.67
Me10xEm1	4	2	50.00	Me1xEm4	5	2	40.00
Me10xEm2	2	1	50.00	Em1xMe3	5	1	20.00
Me9xEm3	4	2	50.00	Me1xEm6	6	2	33.33
Me9xEm4	5	5	100.00	Me1xEm7	7	1	14.29
Me10xEm3	3	0	00.00	Me1xEm8	4	3	75.00
Me10xEm4	6	3	50.00	Me2xEm2	5	1	20.00
Me9xEm6	5	4	80.00	Me2xEm4	10	7	70.00
Me9xEm7	6	3	50.00	Me2xEm6	8	0	00.00
Me10xEm13	7	4	57.14	Me2xEm7	8	0	00.00
Me10xEm6	3	0	00.00	Me3xEm1	9	9	100.00
Me9xEm7	3	2	66.67	Me3xEm3	7	3	42.86
Me9xEm8	8	7	87.50	Me3xEm6	11	10	90.91
Me10xEm7	6	4	66.67	Me4xEm3	6	3	50.00
Me10xEm8	8	3	37.50	Me4xEm4	8	8	100.00
Me9xEm9	8	7	87.50	Me4xEm6	4	3	75.00
Me9xEm10	6	3	50.00	Me4xEm9	6	5	83.33
Me10xEm9	8	7	87.50	Me4xEm10	7	3	42.86
Me10xEm10	6	4	66.67	Me5xEm6	7	3	42.86
Me9xEm11	9	7	77.78	Me6xEm3	5	5	100.00
Me9xEm12	4	4	100.00	Me6xEm6	6	2	33.33
Me10xEm11	0	0	00.00	Me7xEm3	6	1	16.67
Me10xEm12	0	0	00.00	Me8xEm9	7	6	85.71

The genetic similarity of the four clones varied from 0.329 to 0.809 (Figure 2B). Gubbuk et al. (2004) revealed that genetic similarities among Dwarf Cavendish types ranged from 0.550 to 0.913, and the genetic differences ranged from 0.088 to 0.413 as determined by RAPD analysis. Pinar et al. (2015b) reported that Azman was the most diverse clone among the cultivars after Grand Nain. Most of the genotypes showed a low level of variation, and the genetic similarity was over 0.90. Unlike the RAPD analysis, AZ was separated from GN in SRAP analysis. These results were also previously reported for other vegetatively propagated fruit species. And they assumed that morphological differences in that species were mainly due to mutations. In the present study, EY was found to be more identical than others. Comparison between SRAP-RAPD markers and morphological data, three dendrograms based on molecular markers and morphological trait data almost corresponded to each other.

4. Conclusion

The present study revealed that both morphological and molecular markers (RAPD-SRAP) provided consistent information which complemented each other and should be used together for greater clarity in variability and breeding studies of different banana clones. In addition, morphological markers are also suitable for initial screening of clones. Assessment of grouping of banana clones by using the molecular and morphological markers will be useful in the banana breeding programs. Especially, molecular markers can enhance the effectiveness of breeding new and adapted clones in terms of time.

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Conflicts of Interest

No potential conflict of interest was reported by the author(s).

Abbreviations

AZ: Azman

DC: Dwarf Cavendish

EY: Erdemli Yerli

GN: Grand Nain

NTSYS: Numerical Taxonomy Multivariate Analysis System

RAPD: Random Amplified Polymorphic DNA

SRAP: Sequence Related Amplified Polymorphism

UPGMA: Unweighted Pair Group Method with Arithmetic Averages

UPOV: International Union for the Protection of New Varieties of Plants

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